JOURNAL OF DAIRY SCIENCE

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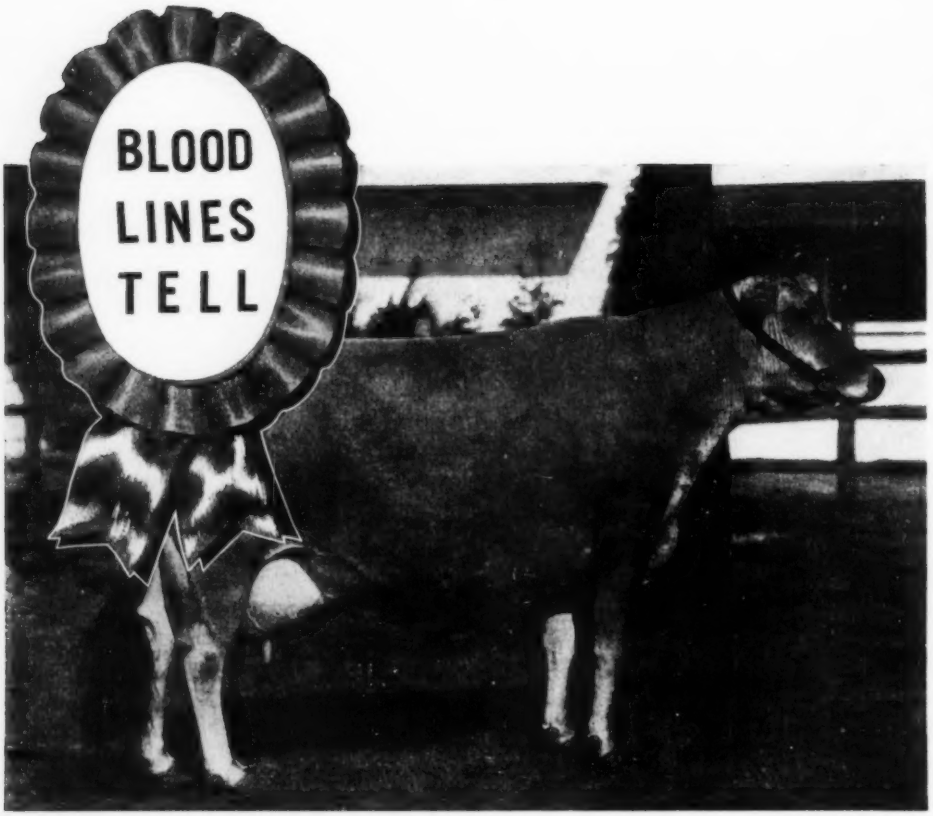
Vol. XXIV, No. 1, January, 1941

Published by the

AMERICAN DAIRY SCIENCE ASSOCIATION



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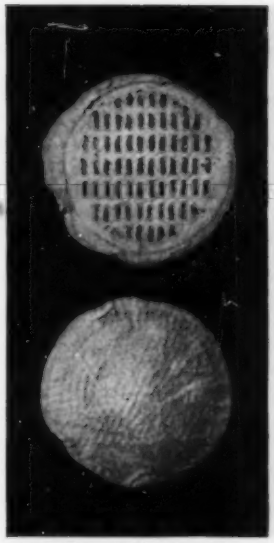

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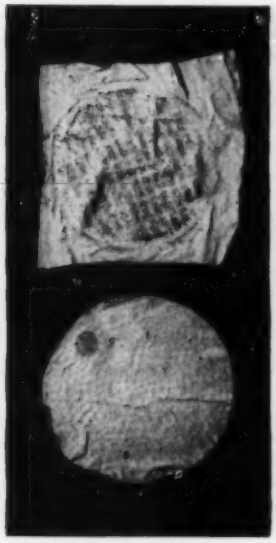

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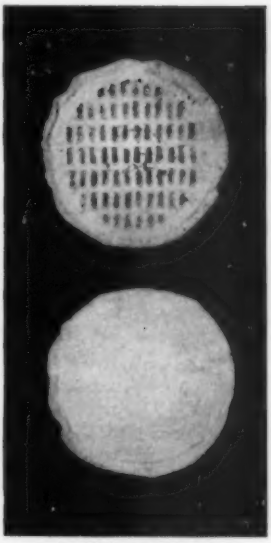

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The Journal of Dairy Science is issued monthly. Subscription is by the volume and one volume is issued per year.

Manuscripts should be typewritten and carefully revised before submission to T. S. Sutton, The Ohio State University, Columbus, Ohio. Twenty-five reprints will be furnished gratis to authors. Cost of additional reprints and reprint order blank will be submitted with proof.

The use of material published in the Journal is encouraged and a liberal policy will be followed concerning reproduction of articles with proper notation as to source.

Subscriptions. Price; $6.00 per volume in United States and Canada; $6.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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Advertising should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Correspondence regarding business policies of the Journal should be addressed to the Secretary-treasurer, R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Post Office Notices of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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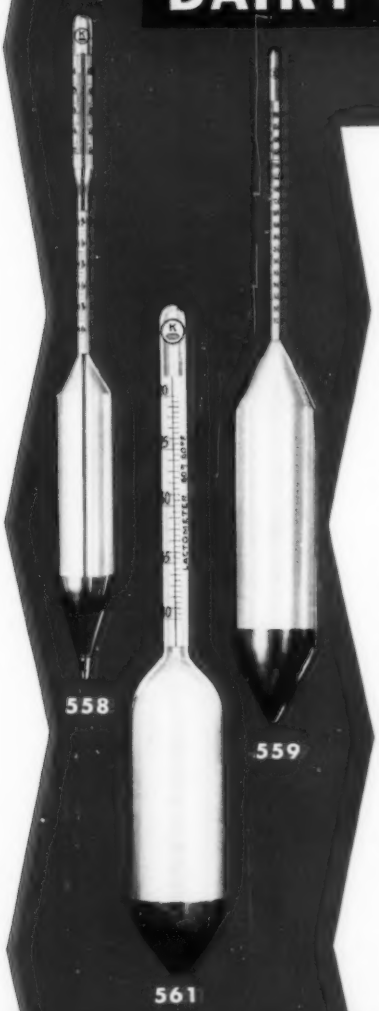

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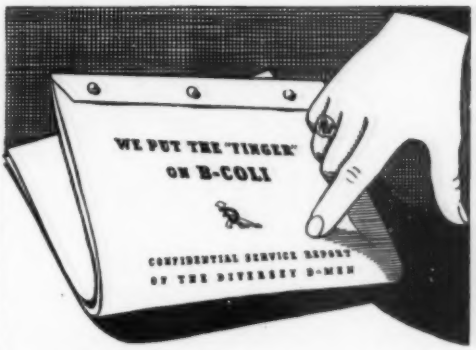

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JOURNAL OF DAIRY SCIENCE

VOLUME XXIV JANUARY, 1941 NUMBER 1

THE EFFECT OF ALFALFA LIPIDS UPON THE PROGRESS OF SWEET CLOVER POISONING IN CATTLE*

W. A. KING, H. A. CAMPBELL, I. W. RUPEL, P. H. PHILLIPS, AND G. BOHSTEDT

Departments of Biochemistry and Dairy Husbandry, University of Wisconsin, Madison

In a previous publication data obtained by Phillips *et al.* (4) seemed to indicate that cattle need added vitamin K on certain diets. On the basis of this preliminary observation an attempt was made to ascertain whether or not vitamin K was associated with toxic sweet clover poisoning.

The investigations of Schofield (9, 10), Roderick (7, 8), and Quick (5) indicate that toxic sweet clover poisoning results in a lowering of the prothrombin content of the blood. Schonheyder (11) and Dam *et al.* (2) and others have shown that a vitamin K deficiency in the chick causes a reduced prothrombin level of the blood. On the other hand, Campbell *et al.* (1) were unable to protect rabbits against toxic sweet clover poisoning with 2-methyl 1; 4 naphthoquinone.

Quick (6) used dehydrated alfalfa meal to protect against toxic sweet clover poisoning and reported the prevention of the delay in clotting time. Smith (12, 13) could not confirm these results even with 50 per cent alfalfa hay in the toxic sweet clover ration of the rabbit.

EXPERIMENTAL

Two Guernsey bull calves were purchased from a farm where several deaths from toxic sweet clover poisoning had occurred in the herd. These calves, which had received toxic sweet clover as a part of their roughage diet for some six weeks before purchase, were placed upon a ration composed of white corn 22 parts, linseed meal 23 parts, wheat middlings 11.5 parts, toxic sweet clover 40 parts, ground limestone 3.0 parts, iodized salt 0.5 part, and irradiated yeast 0.1 part. Carotene in Wesson oil was used to supply vitamin A. These calves weighed about 500 pounds at the beginning of the period. Two Holstein heifers (350 lbs.) were fed a similar ration except that they received only 10 per cent toxic sweet clover in place of the 40 per cent mentioned above. Other cattle of like age and weight were also available as controls.

Received for publication July 22, 1940.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

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TABLE 1

Blood clotting and prothrombin data on the toxic sweet clover fed animals

Days of experiment	Blood clotting time	Prothrombin time	Remarks
	Calf 34		
	min.	*sec.*	
0	4	29	40% sweet clover
9	8	23	
21	43	103	No sweet clover from 21–33rd day
23	24	71	
28	13	29	
44	16	81	40% sweet clover
51	38	104	
			Bile salts added 53–56 days
58	80	92	40% sweet clover + Alf. ext. + b.s. from
60	60	115	58th–60th day
62	50	82	
65	47	50	40% sweet clover
72	42	71	
79	41	62	
93	41	63	
100	31	70	40% sweet clover + Alf. ext. + b.s. from
105	26	48	94–111th day
107	15	48	
112	11	54	
114	20	46	40% sweet clover
121	22	57	
135	30	48	
146	26	59	40% sweet clover + Alf. ext. + b.s. from
149	33	.....	146–165th day
154	27	44	
156	23	36	
161	16	.....	
163	18	35	
167	13	39	
171	21	46	40% sweet clover
177	30	39	
184	11	62	
189	22	46	
196	22	56	b.s. alone for 196–216th day
198	28	46	
205	40	50	
210	19	37	
212	35	43	
216	35	31	
219	20	32	No sweet clover from 216–230th day
223	13	26	
227	12	28	
230	8	29	
	Calf 38		
0	12	34	40% sweet clover
16	13	130	
21	20	110	40% sweet clover + alf. ext. + b.s. from
23	18	140	21–26th day
28	20	79	
30	16	73	

TABLE 1.—(*Continued*)

Days of experiment	Blood clotting time	Prothrombin time	Remarks
	min.	*sec.*	
37	8	25	No sweet clover from 30–44th day
44	8	27	
58	11	60	40% sweet clover
65	28	48	
70	30	60	
79	30	58	
100	24	46	
107	23	43	
112	20	57	
114	19	40	
121	10	47	
128	21	40	
135	16	42	
154	13	32	
161	13	27	
163	10	38	60% sweet clover from 160–194th day
171	14	38	
184	11	42	
189	11	38	
191	13	48	
196	11	37	No sweet clover from 194–203rd day
198	11	23	
203	8	.....	

b.s. = bile salts.

Routine analyses at biweekly intervals, or as needed, included the following: blood clotting time by the capillarly method at 38° C., prothrombin time after the method of Campbell and Link (1), serum calcium, hemoglobin, fibrin, and blood platelets.

Alfalfa extracts were made by extracting good alfalfa hay with crude petroleum ether. The bile salts were obtained commercially. These materials were used therapeutically as indicated.

RESULTS

Repeated observations upon blood clotting time established the fact that normal bovine blood clots between 4 and 8 minutes on the average. In all cases blood which failed to clot within 10 minutes was considered to be pathologic. Normal calves have prothrombin clotting times lying between 25–35 seconds. When the clotting time exceeded 35 seconds it was considered to indicate a lowered prothrombin content in the blood.

The Holstein heifers which received only 10 per cent toxic sweet clover remained normal throughout the experiment as indicated by the analyses. They were therefore discontinued after 121 days on the experiment.

The data on the two Guernsey bull calves are given in table 1. It is evident that during periods of alfalfa extract-bile salts therapy there was a

reduction in both the blood and prothrombin clotting time. It was likewise shown that bile salts were ineffective in bringing about a remedial effect upon the blood clotting time, but it did seem to affect the prothrombin time. These results are graphically shown in figure 1. In this case therapy periods have been taken from the plotted prothrombin and blood clotting curves for the entire period. In order to show the direction of the trends more clearly, lines of regression have been fitted to the data for these periods. On a ration wherein the 40 per cent toxic sweet clover was replaced by oat mill feed complete recovery occurred in a very short time. During the time when the crude petroleum ether extract of alfalfa was given together with bile salts a marked reduction in clotting time occurred in all four therapy periods.

During the periods in which the blood clotting time was prolonged up to 60 and 80 minutes in these animals, the blood platelets rose from a normal of about 300,000 to 350,000 per cu. mm. to as high as 730,000 per cu. mm. The hemoglobin, serum calcium, and fibrin were found to be normal at all times throughout the experiment.

Protocol for calf No. 38. After 21 days on the sweet clover ration (subsequent to a period of some toxic sweet clover feeding prior to purchase for experimental purposes) the prothrombin and blood clotting times were 110 seconds and 20 minutes, respectively. At this time alfalfa extract equivalent to 11 kilograms of alfalfa was given over a two-day period, then extract equivalent to 24 kilograms of alfalfa with bile salts was given for the next three days. Four days later (figure 1) the prothrombin had fallen to 73 seconds and the blood clotting time had dropped to 16 minutes. At this point calf 38 was put on a ration in which oat mill feed replaced the sweet clover hay in the mixed ration and in seven days the calf was back to normal. Shortly after this he was again given the sweet clover ration and the blood clotting time rose to 30 minutes and the prothrombin time to 60 seconds. But from that time on there was a slow fall (table 1) toward normal until levels of about 13 minutes for blood clotting time and 32 seconds for prothrombin time, were reached. After 161 days on experiment the toxic sweet clover hay was increased from 40 per cent to 60 per cent in the ration of this calf. The blood clotting time remained unchanged and the prothrombin time showed only 38–48 seconds.

Protocol for calf No. 34. After 21 days on the toxic sweet clover ration (subsequent to purchase) calf No. 34 showed a blood clotting time of 43 minutes and a prothrombin time of 103 seconds (table 1). At this point he was put on a ration with the toxic sweet clover replaced by oat mill feed (figure 1) and in 7 days the prothrombin was normal and the blood clotting time was 13 minutes. Returning again to the toxic sweet clover ration his blood clotting time soon reached 38 minutes and the prothrombin time rose to 104 seconds. Twenty-three grams of bile salts were given over a period of three days (table 1). There was a drop from 104 to 92 seconds in the pro-

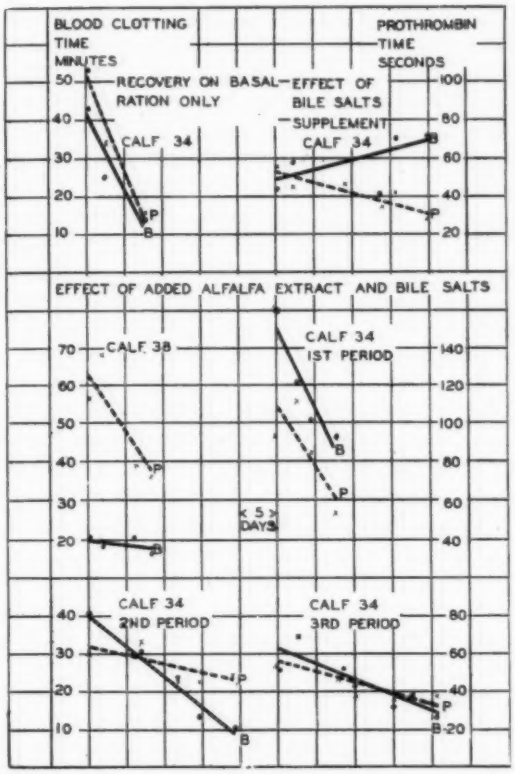

FIG. 1. Therapy of sweet clover poisoned animals. Lines of regression fitted to data. P = prothrombin. B = blood clotting time.

thrombin time. However, the blood clotting time continued to rise quite rapidly. Alfalfa extract equivalent to 20 kilograms of alfalfa hay together with bile salts was then given over a period of two days. There was a steady fall from 80 to 47 minutes in blood clotting time and after a temporary rise from 92 to 115 seconds the prothrombin time dropped to 82 seconds and finally to 50 seconds over a seven-day period (figure 1).

Since these data seemed to indicate that the alfalfa extract plus bile salts was effective in partially correcting the pathologic blood picture of toxic sweet clover poisoning, it was decided to try feeding the extract mixture for longer periods of time. No. 34 in the second test showed at the beginning a blood clotting time of about 40 minutes and prothrombin time of about 63 seconds. Over a period of eight days an extract equivalent to 45 kilograms of alfalfa hay was given with bile salts. There was a three-day interval

without extract followed by the administration of extract equivalent to 34 more kilograms of alfalfa hay with bile salts, given over a seven-day period. A steady drop in the blood clotting time from 40 minutes to 11 minutes and a delayed drop from 63 seconds to about 50 seconds in the prothrombin over the 18 days was obtained (figure 1). The blood clotting time shows a spectacular return to near normal and the prothrombin time dropped about half way to normal.

A third test period was run with calf No. 34 after 34 days had elapsed following the above test. During the interim he was fed the 40 per cent toxic sweet clover ration. At the beginning of this test (table 1) the blood clotting time was 26 minutes and the prothrombin time was 59 seconds. Extract, equivalent to 82 kilograms of alfalfa hay, plus bile salts, were fed over a period of the next 19 days. In the first three days the blood clotting time increased to 33 minutes, followed by a drop to 13 minutes at the end of 21 days. The prothrombin time dropped from 59 to 39 seconds (figure 1).

To determine the effect of bile salts No. 34 was fed bile salts in capsules for a period of 20 days. During this time the prothrombin time dropped from 56 seconds to 31 seconds. The blood clotting time was somewhat erratic but it rose from 22 minutes to 35 minutes (figure 1).

DISCUSSION

In view of the foregoing results it is apparent that crude petroleum ether extracts of alfalfa hay given to growing cattle while they received poisonous levels of toxic sweet clover alleviated the disease. The explanation of these results is not clear. It is possible that the lipid material extracted from the alfalfa carried a factor or factors which were essential to the physiology of the animal in dealing with the toxic principle. On the other hand, it is also entirely possible that the fatty mass (extract) given to the calf in two daily doses may have reduced the absorption of the toxic principle and thereby afforded protection. The latter seems unlikely since the carotenoids of the extract were readily absorbed as indicated by the blood plasma. This would indicate that there was considerable digestion and absorption of the extracted lipids which were fed.

Several results indicate that one or more factors other than prothrombin are involved in the restoration of the blood clotting process in the toxic poisoning caused by improperly cured sweet clover. First, the differences in the trend of the blood clotting time and prothrombin clotting time suggest that the crude petroleum ether extracts of alfalfa supplied an essential constituent in the therapy of sweet clover poisoning. Second, bile salts administration brought about an increase in blood prothrombin as shown by the reduction of the prothrombin clotting time. This might be interpreted to mean that sufficient residual vitamin K, or similar prothrombin stimulating substances were present and the bile salts only facilitated the absorption.

Third, when the toxic sweet clover was completely removed from the ration the prothrombin quickly returned to normal but the whole blood clotting time continued to be delayed.

A test to establish the presence of vitamin K in toxic sweet clover was made. Results with growing chicks fed a vitamin K low ration showed that 15 per cent toxic sweet clover supplied sufficient vitamin K to maintain normal blood clotting properties in this species (3). On the other hand, chicks fed the vitamin K low ration alone developed prolonged whole blood and prothrombin clotting times. However, the alfalfa lipids were required with the bile salts in order to markedly affect the whole blood clotting time in the bovine.

Since bile salts fed alone diminished the prothrombin clotting time, and since toxic sweet clover was shown to contain vitamin K in appreciable amounts (3), it seems unlikely that vitamin K itself is directly concerned with counteracting toxic sweet clover poisoning. These experiments are not crucial in this respect and therefore do not preclude this possibility.

Our results confirm those of Roderick (8) in that there was an increase in blood platelets when the animals showed a distinct reduction in clotting power, but the delay in coagulation time was independent of the increase in blood platelets. It is generally conceded that the blood platelets furnish thromboplastin and if this is true then there was sufficient thromboplastin present in the bloods of the poisoned calves.

SUMMARY AND CONCLUSIONS

Ten per cent of the ration of growing cattle was made up of toxic sweet clover and fed without harm for a period of 3½ months.

Animals with a prolonged clotting time developed an increased number of blood platelets. There was no change in the fibrin, hemoglobin, or serum calcium in these cases.

Crude petroleum ether extracts of alfalfa hay fed at a level equivalent to 60 per cent of the toxic sweet clover in the ration brought about a favorable remedial response in sweet clover poisoned young cattle. Evidence adduced from the separate effects upon whole blood clotting time and prothrombin clotting time, the administration of bile salts alone and with alfalfa lipids, and the difference in rate of return to normal between the prothrombin and blood clotting times when the toxic hay was withdrawn from the ration indicates that one or more factors other than prothrombin were involved in the restoration of the normal blood clotting mechanism of the sweet clover poisoned bovine.

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DISTRIBUTION OF *PSEUDOMONAS FRAGI**

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Pseudomonas fragi is of special significance to the dairy industry because of the off flavors it causes in milk, cream, butter, cottage cheese and other products (1). It is both proteolytic and lipolytic and in addition is psychrophilic so that it can bring about objectionable changes at temperatures commonly used for holding dairy products.

Because of the frequency with which *Ps. fragi* is responsible for spoilage in dairy products its distribution was studied, and the results are reported herein. Most of the samples examined for the organism originated in Iowa, but a number were obtained in Kentucky and some in other states.

ISOLATION PROCEDURE

Collection of samples. Milk samples were collected in sterile, cotton-stoppered test tubes and samples of such materials as butter, water, soil, etc., were collected in sterile, screw-cap, glass jars or bottles. Material for examination was obtained from such possible sources as stanchions, milking utensils, floors, churns, vats, etc., by means of sterile moistened swabs.

Isolation of cultures. In attempting to isolate cultures an enrichment procedure (1) was regularly used. Milk samples, or tubes of sterile litmus milk inoculated with other materials sampled or with swabs used on equipment, floors, etc., were kept at 5° to 10° C. for 7 to 10 days and then plated on beef extract or beef infusion agar to which nile blue sulfate and butter fat emulsion had been added (2). Dilutions used were from 1–10,000 to 10,000,000. Plates were incubated at room temperature (20° to 22° C.) for 2 to 3 days.

Colonies of lipolytic organisms suggestive of *Ps. fragi* were picked into tubes of litmus milk. Characteristic colonies take up very little dye from the medium and appear almost chalk white when examined from the bottom of the plate with a lens. The litmus milk cultures were incubated at room temperature. Usually, after 2 to 3 days cultures of *Ps. fragi* developed a May apple odor and also a pink or acid ring at the surface of the milk next

Received for publication July 22, 1940.

* Journal paper of the Kentucky Agricultural Experiment Station, published by permission of Thomas P. Cooper, Director of the Kentucky Agricultural Experiment Station, and Journal Paper J–783 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 119. The studies in Iowa were financed in part through a grant from the Iowa State Brand Creameries, Inc., of Mason City, Iowa.

to the wall of the tube. Cultures exhibiting characteristics of *Ps. fragi* were later studied in more detail.

While the isolation procedure used is relatively simple and reasonably effective, in some instances it probably fails to detect the organism. Of necessity, the amounts of material examined are small and possibly are not representative. In considering the results reported, it should be recognized that failure to isolate *Ps. fragi* from a sample does not preclude the possibility of its presence.

EXPERIMENTAL

Distribution of Ps. fragi in Iowa

In milk. Samples of milk from the regular supplies delivered to the Iowa State College milk plant from 14 farms were examined for *Ps. fragi* (table 1). From 5 to 21 samples were obtained at intervals from the

TABLE 1

Ps. fragi in milk delivered to the Iowa State College milk plant

Farm No.	No. samples examined	Samples yielding *Ps. fragi*	
		No.	Per cent
1	5	0	0.0
3	15	0	0.0
4	13	2	15.4
5	9	0	0.0
6	20	6	30.0
7	15	2	13.3
8	21	9	42.9
9	6	0	0.0
10	14	2	14.3
11	12	3	25.0
12	12	2	16.7
13	8	1	12.5
14	13	1	7.7
15	13	1	7.7
Summary	176	29	16.5

various supplies. Milk from 10 (71.4 per cent) of the 14 farms yielded *Ps. fragi* in one or more instances. The percentages of samples from the 10 farms that yielded *Ps. fragi* ranged from 7.7 to 42.9 per cent and averaged 18.5 per cent. Of the total 176 milk samples examined, 29 (16.5 per cent) yielded *Ps. fragi.* It was found in 7 (7.7 per cent) of 91 samples obtained in October, in 16 (22.9 per cent) of 70 samples collected in November and in 6 (40.0 per cent) of 15 samples obtained from December to May.

In defective dairy products. In the examination of defective dairy products, 16 (57.1 per cent) of 28 butter samples yielded *Ps. fragi;* the butter samples showed various defects but most of them were rancid. The organism also was isolated from a sample of rancid butter serum, 2 samples

of rancid pasteurized cream and a sample of raw skimmilk which had developed a May apple odor on holding in a refrigerator. Thus, 20 (62.5 per cent) of 32 samples of defective butter and other dairy products yielded *Ps. fragi*. It was isolated from 14 (70.0 per cent) of 20 samples of dairy products described as rancid or having a May apple odor; presumably, these defects were caused by *Ps. fragi*.

In dairy plants. Material from plant equipment was obtained in 39 creameries. Most of the swabs were from churns but a few were obtained from printing and wrapping equipment, vats and miscellaneous pieces. Samples from 60 churns in 37 creameries yielded *Ps. fragi* in only 2 cases (3.3 per cent). Three samples from printing or wrapping tables and a sample from a printer belt each yielded the organism. In all, *Ps. fragi* was found in only 6 (5.8 per cent) of the 104 samples.

In one creamery swabs were obtained from 24 pieces of equipment. These included nearly all the equipment with which cream or butter came in contact. The only sample yielding *Ps. fragi* was obtained from the top of the table on which butter was wrapped.

In a milk plant 20 swabs were obtained from various pieces of equipment and from various locations, such as the weigh room floor and the loading platform. The only sample yielding *Ps. fragi* was from the floor in front of the weigh tank.

Following the isolation of *Ps. fragi* from 2 samples of rancid pasteurized cream, a batch of cream was followed through the processing operations in the plant where the defect occurred. *Ps. fragi* was isolated from the raw cream in the vat before pasteurization was begun. None of the samples taken after the cream had reached the pasteurization temperature (68.3° C.) yielded the organism.

In dairy plant water supplies. Samples of water from 37 creameries were examined. Most of them represented regular water supplies, but 5 were samples of wash water taken from churns when butter was being washed and undoubtedly contained organisms from the butter and churns, 1 was water in which parchment was soaking and 2 were brine used for the same purpose.

Of the 33 samples from regular water supplies, 5 (15.2 per cent) yielded *Ps. fragi*. However, 3 of these samples were from one creamery; therefore, *Ps. fragi* was isolated from the regular water supplies of 3 (9.7 per cent) of the creameries. One (20.0 per cent) of 5 samples of wash water yielded the organism, as did 1 (50.0 per cent) of 2 samples of brine used for soaking parchment. In all, *Ps. fragi* was isolated from 7 (17.1 per cent) of the 41 water (or brine) samples.

On farms. Six of the 14 farms supplying the milk samples examined (table 1) were visited and various samples obtained. Two of the farms

to the wall of the tube. Cultures exhibiting characteristics of *Ps. fragi* were later studied in more detail.

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		No.	Per cent
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3	15	0	0.0
4	13	2	15.4
5	9	0	0.0
6	20	6	30.0
7	15	2	13.3
8	21	9	42.9
9	6	0	0.0
10	14	2	14.3
11	12	3	25.0
12	12	2	16.7
13	8	1	12.5
14	13	1	7.7
15	13	1	7.7
Summary	176	29	16.5

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Of the 33 samples from regular water supplies, 5 (15.2 per cent) yielded *Ps. fragi*. However, 3 of these samples were from one creamery; therefore, *Ps. fragi* was isolated from the regular water supplies of 3 (9.7 per cent) of the creameries. One (20.0 per cent) of 5 samples of wash water yielded the organism, as did 1 (50.0 per cent) of 2 samples of brine used for soaking parchment. In all, *Ps. fragi* was isolated from 7 (17.1 per cent) of the 41 water (or brine) samples.

On farms. Six of the 14 farms supplying the milk samples examined (table 1) were visited and various samples obtained. Two of the farms

(farms 6 and 8) supplied the milk from which *Ps. fragi* had been isolated most frequently. On the first visit to these farms the organism was found in 8 (38.1 per cent) of 21 samples from farm 6 and in 13 (65.0 per cent) of 20 samples from farm 8. The samples which yielded the organism at both farms were dirt from the barn floor, bedding, milk (not aseptically drawn) and swabs from milk pails, milk stools and ledges. In addition, at farm 6 it was found in the grain mixture and surface water from the barnyard, and at farm 8 it was isolated from water in a stock watering tank, dirt from the barnyard, hay and swabs from coats of cows, a stanchion and a floor scraper handle. On the second visit to each of the farms *Ps. fragi* was isolated from 9 (69.2 per cent) of 13 samples obtained at farm 6 and from 6 (50.0 per cent) of 12 samples obtained at farm 8. The samples which yielded the

TABLE 2

Ps. fragi in samples from six Iowa farms

Type of sample	No. samples examined	No. samples yielding *Ps. fragi*	Totals		
			No. samples examined	Samples yielding *Ps. fragi*	
				No.	Per cent
Water from					
tap	3	0			
stock tank	11	8			
barn floor	1	1	19	11	57.9
cooling tank	1	0			
soil surface	3	2			
Dirt from					
barnyard	12	6	14	8	57.1
barn floor	2	2			
Feed					
hay	8	5			
grain	8	3	17	8	47.1
silage	1	0			
Bedding	9	8	9	8	88.9
Animals					
coat	9	3	15	5	33.3
milk	6	2			
Milking utensils					
milking machine	4	0			
pails	4	2			
strainers	2	0	14	2	14.3
cooler	1	0			
cans	3	0			
Misc. barn equipment					
stanchions	15	8			
milk stools	9	5	29	16	55.2
scrapers, shovels, etc.	5	3			
Floors	10	6	19	12	63.2
Ledges	9	6			
Manure	1	1	1	1	100.0
Summary			137	71	51.8

organism at both farms were bedding and swabs from the barn floor, a stanchion and dusty ledges. At farm 6 samples of water in a stock watering tank, barnyard dirt, hay and swabs from the coat of a cow and a milk stool also yielded *Ps. fragi*, while at farm 8 a swab from a floor scraper handle also yielded it. *Ps. fragi* was isolated from 36 (54.5 per cent) of 66 samples obtained on the two farms.

Five visits were made to farms from whose milk supplies *Ps. fragi* had not been isolated. On the first visit to farm 1, 12 (50.0 per cent) of 24 samples yielded the organism and on the second visit 5 (41.7 per cent) of 12 samples contained it. Samples from this farm that yielded *Ps. fragi* included water in a stock watering tank and on the barn floor, barnyard dirt, hay, bedding and swabs from stanchions, floors and manure. On each of the other three farms *Ps. fragi* was isolated from samples of barnyard dirt and bedding. On two of the three farms water in stock watering tanks and swabs from stanchions, milk stools and ledges also yielded it. Samples from which it was isolated on only one of the three farms included surface water, hay and swabs from a floor scraper handle and a dusty ledge. The numbers of samples examined from the three farms were 11, 12 and 12, and the percentages of these samples yielding *Ps. fragi* were 54.5, 58.3 and 41.7 per cent, respectively. In all, 71 samples obtained on the four farms yielded *Ps. fragi* in 35 (49.3 per cent) of the instances.

A summary (table 2) of the results of examinations of samples obtained on the nine visits to the six Iowa farms shows that of the materials and equipment sampled bedding, floors and ledges, water, dirt, miscellaneous barn equipment, feed, the cows and milking utensils harbored *Ps. fragi* in the order named.

Distribution of Ps. fragi in Kentucky

In milk. Three sets of samples from milk delivered to a Lexington, Kentucky, milk plant were examined for *Ps. fragi* (table 3). One set was

TABLE 3

Ps. fragi in milk delivered to a Lexington, Kentucky, milk plant

	No. samples examined	Samples yielding *Ps. fragi*	
		No.	Per cent
6–27–39	17	0	0.0
12– 6–39	20	8	40.0
12–22–39	20	8	40.0
Summary	57	16	28.1

obtained in June and two sets in December. The samples represented the supplies of 26 farms in three counties. Six farms supplied 1 sample each, nine farms 2 samples each and eleven farms 3 samples each.

None of 17 samples obtained in June yielded the organism, but it was found in 16 (40.0 per cent) of 40 samples obtained in December. Including summer and winter samples, *Ps. fragi* was isolated from 16 (28.1 per cent) of the 57 samples.

Of the 17 farms represented by the June samples, only 12 were represented by the December samples. *Ps. fragi* was found in samples from 9 (75.0 per cent) of the 12 farms. In all, it was isolated from samples representing the milk supplies of 13 (50.0 per cent) of the 26 farms.

In defective dairy products. Each of 2 samples of skimmilk which developed a May apple odor in a refrigerator yielded *Ps. fragi.* It was not isolated from 7 samples of butter following keeping quality tests; none of the samples were criticized as being rancid or having a May apple odor.

In dairy plants. A swab from a churn did not yield *Ps. fragi.* It was isolated from 2 swabs obtained in June and December from the receiving room floor of a plant. In June it was not obtained from the floor of a covered truck delivering milk to this plant, while in December a similar sample yielded the organism.

In water. A sample of pond water received from a farmer who questioned its fitness for cattle to drink contained *Ps. fragi.* Water, including some milk solids, that remained in a shipping box after removal of milk and cream samples developed a May apple odor and *Ps. fragi* was readily isolated from it.

Distribution on farms. Forty-nine samples representing various materials and sources were obtained on 10 farms during the summer; most of them were collected in July and August when the weather was hot and dry. Only 2 (4.1 per cent) of the samples yielded *Ps. fragi.* Both were samples of feed, 1 of grain and 1 of hay.

Ninety-nine samples were obtained from nine farms in December. Of these 37 (37.4 per cent) yielded *Ps. fragi.* It was isolated from 9 of 12 water samples (0 of 13 in summer), from 11 of 14 dirt samples (0 of 12 in summer), from 3 of 18 feed samples (2 of 19 in summer), from 3 of 6 samples of bedding (no tests in summer), from 0 of 9 samples from utensils (no tests in summer), from 4 of 21 samples from barn equipment (0 of 3 in summer) and from 7 of 19 samples from floors and ledges (0 of 2 in summer).

Samples were obtained from only one of the farms in both summer and winter. Of 14 samples collected on the farm in June, only 1 (grain) yielded *Ps. fragi,* while it was found in 7 of 11 samples obtained in December. The winter samples from which it was isolated were water (2 samples), dirt (2 samples), bedding and swabs from the floor and from a dusty ledge. Approximately the same materials and equipment were sampled on each visit.

A summary (table 4) of the results of examinations of all samples obtained on the 18 Kentucky farms shows that *Ps. fragi* was found in 39 (26.4 per cent) of the 148 samples and that bedding, dirt, water, floors and ledges, miscellaneous barn equipment and feed harbored the organism in the order given. One-third to one-half of the samples in the first four groups yielded *Ps. fragi*, but it was not found in the samples from milking utensils.

TABLE 4

Ps. fragi in samples from 18 Kentucky farms

Type of sample	No. samples examined	No. samples yielding *Ps. fragi*	Totals		
			No. samples examined	Samples yielding *Ps. fragi*	
				No.	Per cent
Water from					
stock tank	11	5			
drinking cup	3	1			
creek	1	1			
cistern	1	1			
tap	1	0	25	9	36.0
cooling tank	3	0			
wash tank	1	0			
barn floor	2	0			
rinse from utensils	2	1			
Dirt from					
barnyard	19	9			
barn floor	4	1	26	11	42.3
manger	2	1			
gutter	1	0			
Feed					
grain	20	2			
hay	16	3	37	5	13.5
silage	1	0			
Bedding	6	3	6	3	50.0
Milking utensils					
milk pails	3	0			
strainer	1	0			
cooler	2	0	9	0	0.0
can	1	0			
bottler	1	0			
bottle	1	0			
Misc. barn equipment					
stanchions	10	1			
milk stools	6	2			
scraper, broom and shovel handles	4	1	24	4	16.7
manger	2	0			
drinking cup	1	0			
wash tank (dry)	1	0			
Ceiling	1	0			
Floors	7	4	21	7	33.3
Ledges	11	3			
Cobwebs	2	0			
Summary			148	39	26.4

The results are similar to those obtained in Iowa, although the percentages of Kentucky samples yielding *Ps. fragi* are somewhat lower. A partial explanation of this may be that approximately one-third of the Kentucky samples were obtained in the summer season, when *Ps. fragi* was found only very infrequently, while all the Iowa farm samples were obtained during the winter and spring.

Distribution of Ps. fragi in the United States

A request for a sample of barnyard soil was sent to each of the state agricultural colleges. Barnyard soil was requested because of the frequency with which *Ps. fragi* had been isolated from it and the convenience of collection and shipment. The requests were made in the late winter or early spring which seemed a favorable period for easy detection of the organism. Samples were received from 35 states, representing all sections of the United States.

Ps. fragi was found in the samples from 25 (71.4 per cent) of the 35 states (table 5). The 10 samples which failed to yield the organism were re-examined with the same results. By grouping the states into an eastern and a western division, an interesting comparison can be made. In the eastern section are included the states from Minnesota to Louisiana and east; in the western section are included those from North Dakota to Texas and

TABLE 5

Ps. fragi in barnyard soil from various states

	All states	States in	
		Eastern division	Western division
No. of states	48	31	17
No. of states represented by samples	35	22	13
No. of samples yielding *Ps. fragi*	25	20	5
Per cent of samples yielding *Ps. fragi*	71.4	90.9	38.5

west. The eastern section included 31 states, and samples were available from 22 of them. *Ps. fragi* was demonstrated in samples from 20 (90.9 per cent) of the 22 states. This indicates a wide distribution of the organism throughout the eastern states. In the western section are included 17 states, and samples were available from 13 of them. Samples from only 5 (38.5 per cent) of the 13 western states yielded *Ps. fragi*. While a single sample from a particular spot is by no means representative of an entire state, nevertheless, the number of samples from this section which did not yield *Ps. fragi* suggests that the organism is not as prevalent in the western states as in the eastern.

DISCUSSION OF RESULTS

The results indicate that in Iowa and Kentucky milk, as delivered from the farms, rather commonly contains *Ps. fragi*. Under these conditions

defects caused by the organism are to be expected, unless milk and cream are efficiently pasteurized and these products, or products made from them, are protected against recontamination.

Isolation of *Ps. fragi* from many samples of dirt in seasons favorable for its growth indicates that it is commonly present in soil. Failure to isolate it from 12 such samples obtained on Kentucky farms in summer emphasizes its psychrophilic nature. Since the dirt samples were from the surface and winter samples from some of the same locations yielded the organism, it is probable that in summer it survives deeper in the soil where temperatues remain relatively low. None of the samples of water direct from wells on farms in Iowa or Kentucky yielded *Ps. fragi,* but water from sources exposed to contamination by dirt, such as stock watering tanks, surface of ground, etc., often harbored it. Since bedding, general barn equipment, feed and the animals have ample contact with dirt, the presence of the organism on them is easily explained. Of the dairy equipment examined, only milking utensils are given such care as to control this organism. This is reflected in the data, since only 2 of 23 samples from such equipment yielded *Ps. fragi.* These two samples came from farms whose milk supplies frequently contained the organism, and presumably these dairymen were not caring for their milking equipment as well as some of the others. The frequent presence of *Ps. fragi* in the milk from a farm may indicate conditions which permit contamination from various sources.

The general results suggest that *Ps. fragi,* like various other species of the genus *Pseudomonas,* is a soil organism.

The more frequent occurrence of *Ps. fragi* in barnyard soil in the eastern part of the United States than in the western part may be related to the weather, especially the rainfall. Various states in the western prairie section and the Rocky Mountains have only a comparatively small rainfall, much less than those in the eastern section. In addition, the high temperatures occurring in summer throughout much of the territory probably are detrimental to this psychrophilic organism. These conditions may influence the existence or survival of an organism, such as *Ps. fragi,* whose habitat appears to be soil. Conceivably, such a comparatively dry area might form somewhat of a barrier to the westward spread of *Ps. fragi.*

SUMMARY

Pseudomonas fragi was found in 16.5 per cent of 176 lots of milk delivered by 14 producers to an Iowa milk plant. It was not detected in 17 lots delivered in June to a Kentucky milk plant but was found in 40.0 per cent of 40 deliveries in December to the same plant. Samples of defective dairy products, especially those criticized as rancid or as having a May apple odor, commonly yielded the organism.

In general, dairy plant equipment was relatively free of the organism.

An appreciable percentage (9.7 per cent) of the Iowa dairy plant water supplies yielded the organism.

In Iowa a large proportion (51.8 per cent) of samples of dirt and other materials or equipment likely to come in contact with or be contaminated by dirt was found to harbor *Ps. fragi*. In Kentucky relatively few (4.1 per cent) similar samples obtained in summer yielded it, but it was found in a considerable proportion (37.4 per cent) of the samples obtained in December.

Ps. fragi was found in 25 (71.4 per cent) of 35 samples of barnyard soil obtained from various states. It was present in a larger percentage of samples from the eastern states (90.9 per cent) than from the western states (38.5 per cent).

The wide distribution of *Ps. fragi* on farms emphasizes the importance of farms as a source of the organism.

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THE INFLUENCE OF TEMPERATURE AND VARIOUS COVERINGS ON THE CURING OF FIVE-POUND PRINTS OF CHEDDAR CHEESE*

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INTRODUCTION

The curing of cheddar cheese is of great economic significance to the industry. Changes in methods of curing, merchandizing, size of cheese, and types of coverings have been continuous for nearly fifty years. During the period from 1897 to 1900 the question of ripening cheese at temperatures from below freezing to normal room temperatures was studied extensively. The experiment stations of Wisconsin (1–9), published many separate reports as well as a report in cooperation with the U. S. D. A. (10) on many phases of the problem. Iowa (11) and Ontario Agricultural College (12, 13) also reported extensive experiments on the subject. All the studies gave very similar results with the general conclusions that: (1) the loss of moisture was less at low temperatures, (2) the commercial-quality cheese cured at low temperatures is better, (3) cheese can be held a longer time at low temperatures without impairment of quality, and (4) that by utilizing the combination of paraffining cheese and curing it at low temperatures the greatest economy can be effected.

With the use of lower temperatures for curing, a higher degree of saturation of the atmosphere is always found, which greatly promotes the development of mold. In an effort to control this condition the dipping of cheese in melted paraffin was found satisfactory by two stations (10, 12). This, however, raised the question of its effect upon the development of flavor. It was concluded by the same workers that little injurious effect was produced from the use of paraffin.

The fact that weight loss is experienced when paraffin is used as a coating would indicate that it does not seal the package. Methods of packaging such as the sealed cans recommended by Rogers (14) and the use of cellophane and paraffin as reported by McCubbin (15) present a question of the effect of more nearly sealing the package upon the bacterial activity and the flavor development in the cheese.

The work of Hastings, Evans and Hart (16), as well as that of Dean, Harrison and Harcourt (13), indicates that the kind of active organisms present is more important than the numbers of organisms during the ripening process, and also that the action of rennin and galactase continue at low temperatures after the numbers of organisms in the cheese decline.

Received for publication July 22, 1940.

* Published with the approval of the Director as Paper No. 267, Journal Series, Nebraska Agricultural Experiment Station.

The partial sealing of a cheese would be expected to influence the type of organisms which would be active during ripening. The characteristics of the cheese would also be expected to be effected by this factor as well as the flavor. In an effort to study the effect of several coatings upon five-pound prints of cheddar cheese the following experimental work was carried on.

EXPERIMENTAL PROCEDURES

Five batches of cheddar cheese were made (December 20, 22, 24, 27, 29, 1937) according to what is generally known as the Wisconsin method. Five-pound prints were taken from each batch and covered with the seven materials used in this study: that is, paraffin (Standard Oil Company sheet paraffin), wax (obtained from Candy & Company, Chicago), plain, red, black, orange, and yellow parafilm, as manufactured by the Marathon Paper Mills Company. The paraffin and wax were applied immediately after the cheese had been removed from the press and the surface had dried, by dipping the cheese in the wax or paraffin at 170–175° F. The parafilms were applied after the curd had been pressed for 1 hour without cloths. The prints were then wrapped in parafilm, a heavy brown crepe paper and a press cloth and returned to the press over night. Then the paper and the press cloth were removed and the cheese placed in storage. Thus, prints from each batch with each type of covering material were obtained. Two of these were placed in a 45° F. storage room and two were placed in a 65° F. storage room with approximately 60 per cent relative humidity. One print of each series in each cooler, except the red, orange and yellow parafilm was weighed at 30-day intervals, while a slice was cut from the other print for scoring at monthly intervals. Each of the five batches was analyzed for moisture and fat by the Mojonnier method when the cheese was made.

There was a normal variation in the milk and procedure of making as would be encountered under commercial conditions. No effort was made to standardize procedures, as a variation in the types of cheese was thought to be advantageous rather than disadvantageous in studying the behavior of the cheese as influenced by storage temperatures and covering materials. All batches were made from whole milk pasteurized at 145° F. for 30 minutes and cooled to a setting temperature of 86° F. Color was added at the rate of $1\frac{1}{8}$ oz. per 1000 pounds of milk, rennet at the rate of $3\frac{1}{2}$ oz., and salt at the rate of two pounds per 1000 pounds of milk. Two per cent of fresh lactic starter was used in each batch. Details of the making process will be found in table 1.

THE INFLUENCE OF TEMPERATURE AND COVERING ON QUALITY OF CHEESE

The general characteristics of the different batches of cheese when fresh can be visualized by studying table 1. In general, No. 27 was a sweet curd, low acid type of cheese with mild flavor and a weak and pasty body and No.

TABLE 1

Showing make record and analysis of cheese

Batch No.	Different steps in making cheese												Analysis		Yield per 100 lbs. milk—lbs.
	Acidity			Time required in minutes											
	Milk—% lactic	When dipped % lactic	When milled % lactic	Starter added to setting	Setting to cutting	Cutting to start of heating	Heating time	Heated to dipping	Dipping to milling	Milling to salting	Salting to hooping	Total time	Moisture %	Fat %	
27	.18	.14	.68	4	38	8	70	25	300	45	15	505	37.50	32.50	10.45
29	.18	.20	.87	20	35	10	70	35	90	45	20	325	39.41	35.07	11.00
31	.19	.16	.71	20	35	5	45	40	265	30	20	460	38.56	33.05	11.30
32	.18	.15	.70	25	35	10	50	40	230	30	30	450	39.58	32.60	10.50
33	.17	.16	.72	30	35	10	55	40	175	30	30	405	38.82	33.25	10.90

TABLE 2

Condition of cheese after storage 270 days

		Different batches stored at 45° F.					Different batches stored at 65° F.				
		27	29	31	32	33	27	29	31	32	33
Paraffin	Flavor	Fair	Sl. bitter	Fine	OK	V. sl. fermented	Sl. fermented	Sl. acidy	OK	OK	Sl. unclean
	Body	Sl. open Sl. pasty Weak	Dry Crumbly	OK	OK	OK	Swiss eyes Pasty Weak	Crumbly	OK	OK	OK
	Color	OK	Faded	OK	OK	OK	OK	Faded	OK	OK	OK
	Package	OK	OK	OK	OK	OK	Bad shape	OK	OK	OK	OK
Wax	Flavor	Fair	Sl. bitter	Fair	OK	V. sl. unclean	Sl. fermented	Sl. acidy	Fair	Fair	Sl. unclean
	Body	Sl. open Sl. pasty Weak	Dry Crumbly	OK	OK	OK	Swiss eyes Pasty Weak	Crumbly	OK	OK	OK
	Color	OK	Faded	OK	OK	OK	OK	Faded	OK	OK	OK
	Package	OK	OK	OK	OK	OK	Bad shape	OK	OK	OK	OK

TABLE 2.—(*Continued*)

		Different batches stored at 45° F.					Different batches stored at 65° F.				
		27	29	31	32	33	27	29	31	32	33
White parafilm	Flavor	Fair	Bitter	Fair	Fair	Sl. unclean	Fermented 150 days Unsalable	High acid Fruity	Fruity Unclean	Fruity Fermented	Unclean Fermented-Unsalable
	Body	Sl. open Sl. pasty Weak	Dry Crumbly	Sl. weak	OK	OK	Large Swiss eyes	Crumbly	Sl. open Pasty	OK	Weak Open Pasty
	Color	OK	Faded	OK	OK	OK	Objectionable	Faded	OK	OK	OK
	Package	OK	OK	OK	OK	OK	Wrinkled Bad shape Unsalable	OK	OK	OK	OK
Black parafilm	Flavor	Fair	Bitter	Fair	Fair	Sl. unclean	Fermented 150 days Unsalable	High acid Fruity	Fruity Unclean	Fruity Fermented	Unclean Fermented-Unsalable
	Body	Sl. open Sl. pasty Weak	Dry Crumbly	Sl. weak	OK	OK	Large Swiss eyes	Crumbly	Sl. open Pasty	OK	Weak Open Pasty
	Color	OK	Faded	OK	OK	OK	Faded	Faded	OK	OK	OK
	Package	OK	OK	OK	OK	OK	Wrinkled Bad shape Unsalable	OK	OK	OK	OK

29 was a high acid type with a dry crumbly body. The other three batches were mild in flavor, showing a good body with a tendency to be very slightly open.

A study of table 2 and figures 1 and 2 will show great variation in the effect of storage for 270 days upon the quality of the cheese. It will be observed that batch 27 showed marked development of Swiss eyes and fruity flavor even as early as 90 days at 65° F. as shown in figure 1. This defect

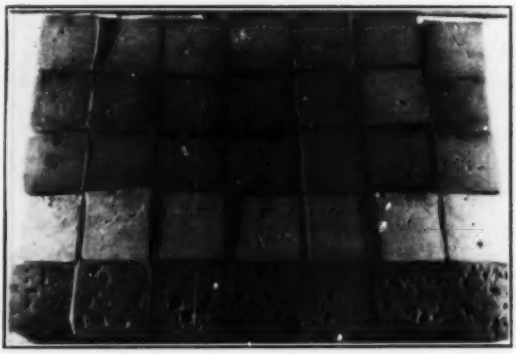

FIG. 1. Five batches of cheese (27, 29, 31, 32, 33) numbered 1, 2, 3, 4, and 5 in that order, at 65° F. at 90 days covered (from left to right) with paraffin, plain parafilm, red parafilm, black parafilm, wax, orange parafilm, and yellow parafilm.

FIG. 2. The same cheese at the same age with the same coverings but held at 45° F.

was much more pronounced in the case of parafilm cheese than the other coverings. Evidently the sweet curd type of cheese in this batch was very favorable for the development of this type of defect. Batch 29, while showing an acid character, did not show Swiss eye development. The other three batches which showed normal body were very slightly open and all developed

some fruitiness in flavor after 270 days at 65° F. but did not show marked Swiss eye development.

It is evident that the making process of the cheese as well as the covering and storage temperature will influence the type of organism which develops during the curing process.

Another quality factor which occurs in dairy products is spoken of as surface taint.

Objectionable surface flavor has occasionally been found on parafilm-covered cheese on the open market. Each of these batches was carefully examined for the presence of this flavor defect. It was not very generally noticeable during the early period of storage at either temperature, but made its appearance at the 45° F. storage temperature after 220 days. Its absence at the higher temperature was probably due to the fact that the flavor defects generally found in that cheese were sufficient to cover any surface taint that might be present. Where it was found in the parafilm covered cheese cured at the lower temperature it was confined entirely to the surface but produced a rather objectionable condition.

THE INFLUENCE OF TEMPERATURE AND COVERING ON WEIGHT LOSS

The weighing of the five-pound prints covered with paraffin, wax and white and black parafilm from the several batches at 30-day intervals produced some interesting results regarding weight loss during storage at 45° F. and 65° F. The results of these weight studies are summarized in table 3.

TABLE 3

Effect of covering on shrinkage during storage

Batch number	Type of covering			
	Paraffin	Wax	White parafilm	Black parafilm
	Shrinkage during 270 days storage at 45° F.			
	%	%	%	%
27	12.75	6.39	2.10	1.04
29	19.27	14.03	1.42	.53
31	10.51	3.55	.91	.93
32	8.28	5.33	.91	.30
33	10.57	5.28	.56	.56
Average	12.42	6.84	1.19	.63
	Shrinkage during 270 days storage at 65° F.			
	%	%	%	%
27	16.25	8.78	4.74	3.80
29	27.17	14.58	11.81	2.79
31	8.66	5.66	9.88	4.39
32	14.60	8.13	11.45	4.76
33	14.17	11.53	5.67	2.19
Average	16.23	9.77	8.69	3.44

The weight loss was greatest in the high-moisture, high-acid batch, No. 29. The other batches of cheese showed considerable variation depending upon the condition under which they were stored and type of covering.

A study of table 3, which also shows the per cent weight loss on the five batches of cheese stored at two temperatures with the different coverings brings out the following facts: (1) The weight loss was decidedly greatest on the paraffin-covered cheese at both temperatures. (2) The weight loss was least on the black-parafilm-covered cheese at both temperatures. (3) The white-parafilm-covered cheese and the wax-covered cheese showed little difference in weight loss at 65° F., but at 45° F. the weight loss was decidedly less in the parafilm-covered cheese than in the wax-covered cheese. The weight losses were consistently less regardless of the covering used when the cheese was ripened at 45° F. than when it was ripened at 65° F.

SUMMARY AND CONCLUSIONS

The paraffin-covered cheese aged uniformly better than the cheese with the comparatively air-tight coverings of the various types of parafilm. Most parafilm-covered cheese eventually developed fruity, fermented flavor defects. These were particularly noticeable at the 65° F. temperature. The cheese aged at the 65° F. temperature with parafilm coverings also showed considerable moisture accumulation under the covering and surface deterioration as the aging process progressed. With the exception of the first few days, the cheese ripened at the 45° F. temperature, regardless of the covering used, was of better quality. Defects in body, particularly those such as Swiss-eye formation, pastiness, and weakness, were much more noticeable in the cheese covered with parafilm than those covered with wax or paraffin.

Surface taint was not generally noticeable with the parafilm covered cheese during the early period of storage at either temperature but made its appearance at the 45° F. temperature after 220 days. It was one of the rather objectionable features encountered in the use of parafilms.

Weight losses were greatly reduced by the use of all parafilm coverings and practically eliminated in the case of 45° F. curing conditions with normal bodied cheese. The black parafilm seemed to have a slight advantage over the white parafilm in this respect.

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ANTIOXYGENIC FRACTIONS OF OAT AND SOYA BEAN FLOUR*

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Investigations of the mechanism of fat oxidation led to the discovery of certain substances which retard the process and aid somewhat in establishing the chemical reactions involved. Most of these substances used earlier are toxic when ingested in large quantities, yet they have found use in protecting from oxidation fatty materials used for purposes other than food. In recent years, edible antioxidants have been used in certain food products. Two such antioxidants are oat flour and soya bean flour, both of which have been used in studying the oxidized flavor of certain dairy products. This flavor defect is believed to be due to the oxidation of the fatty material and is successfully inhibited by either of these antioxidants. The relatively large amount of antioxidant often necessary may produce certain undesirable effects in the dairy product. Consequently, a more concentrated form of the antioxidative substance would be desirable. The antioxidants used in the fat and oil industry include such substances as phospholipids, the unsaponifiable fraction of fat-solvent extracts of many vegetables, gums, and many water-soluble substances. It was thought that some such substance would be mainly responsible for the antioxygenic power exhibited by oat flour and soya bean flour toward the oxidation of milk fat.

REVIEW OF LITERATURE

The process of autoxidation of fat can be divided into two periods; first, the induction period of very slow oxygen absorption, and second the period of active or rapid oxygen absorption. The shortness of the induction period shows a marked correlation to the ease with which various samples of edible fats become tallowy flavored. Recent investigations (7, 8, 19) indicate that the induction period becomes progressively shorter as the substance is purified, and that the induction period is due, in the major part at least, to the presence of traces of foreign substances. According to this theory the prooxidant effect of the products of fat oxidation (activated molecules) will be largely an indirect one resulting from their destructive action on these foreign substances. Moureau and Dufraisse (14) are credited with being the pioneers in the study of these foreign substances, variously termed inhibitors, inhibitols, antioxidants, or antioxygens. However, various substances have for many years been empirically used to preserve fats. Lea (11) mentions the wide use of gum benzoin in preserving lard for pharmaceutical purposes since Deschamps discovered its protective quality in 1843.

Received for publication July 27, 1940.

* Authorized for publication on July 5, 1940, as paper No. 975, in the Journal Series of The Pennsylvania Agricultural Experiment Station.

One theory (11) of the chemical mechanism of antioxidative action is represented by the following scheme where F^* represents an activated molecule of reactant, and A represents the antioxidant molecule.

$$F^* + A \rightarrow F + A^* \qquad A^* + O_2 \rightarrow AO_2$$

When an activated molecule comes into contact with a molecule of the antioxidant, the latter takes up the energy, and as a result is usually but not invariably, oxidized itself by subsequent collisions with oxygen. It fails, however, in its turn to activate any further molecules of the autoxidizing substance, which breaks the reaction-chain. Thus, the effect of the antioxidants upon the velocity of oxidation depends upon whether the energy is more efficiently transferred to the antioxidant or to the reactants.

Another theory (7) suggests that the antioxidant breaks the chain by destroying the moloxides and peracids, having little or no effect on the peroxides.

When all of the antioxidant has been transformed into inert products (that is, at the conclusion of the induction period) the oxidative reaction proceeds at its normal but very high rate, thus indicating that the antioxidant may not be a true anticatalyst.

The specific chemical structure responsible for antioxygenic activity seems to be uncertain although such groups as carboxyl, hydroxyl, and amino, have been suggested as the important factors in certain substances. There is also evidence that certain substances which are insoluble in fat solvents or decomposed by alkali may assist in protecting fats in the tissues.

The antioxygenic value of a substance has been found to disappear entirely when it is employed in a slightly different system. For example, polyhydric phenols are powerful antioxidants in dry fats but lose their activity when the fat is treated with water or mixed with any material containing moisture and protein; and acid inhibitors are powerful in vegetable oils but only slightly if at all active in lard and purified fatty acids. Many similar cases could be mentioned.

The use of oat flour as an antioxidant was first suggested by Musher (15, 16) who reported that it inhibited the oxidation of lard and other fats. The addition of oat flour to ice cream or milk in concentrations varying from one-tenth to five-tenths per cent has been found (4, 12) to prevent or delay the development of either copper-induced or spontaneous oxidized flavor. The addition of twenty-five to thirty per cent oat flour to the paraffin used for coating paper milk bottles was found (4) to prevent or delay either copper-induced or sunlight-induced oxidized flavor in the milk placed in the container. Parchment wrappers treated with oat flour as a sizing have been found (2, 10) to delay staleness in butter. An aqueous extract of oat flour has also been found (1, 3) to protect butter against tallowiness and other off-flavors. Koenig (10) reported that "avenol" (the hexane extract of oat flour) inhibits tallowiness and rancidity in butter. It would therefore seem

that at least part of the antioxidative properties of oat flour is due to substances soluble in fat solvents.

Another vegetable material, soya bean flour, has been reported (15, 16) to have definite antioxygenic powers particularly in animal fats, although it has not been extensively applied to dairy products and milk fat.

GENERAL PROCEDURE

Dry, fresh milk fat was obtained from fresh sweet cream that was known to be free from spontaneous oxidized flavor and to be free from any metal contamination. This fresh cream was churned in a glass churn, the butter transferred to a separatory funnel and washed with hot (71° C.) clean water until the wash water was clear, which required three or four washings with a volume of water equal to the volume of butter each time. The hot fat was then transferred to centrifuge tubes and centrifuged for five minutes at 3500 r.p.m. to dry the fat. The dry, milk fat was then decanted and used or, if necessary, stored at 4.5° C. in the dark until the following day.

The phospholipid fractions of the oat flour and soya bean flour in each case were prepared by the same procedure used by Josephson and Dahle (9) in preparing milk and egg phospholipids. The aqueous extracts were prepared as follows: Oat flour, or soya bean flour, was mixed to a smooth paste with water and diluted with hot water to give an eight per cent mixture at a temperature of 57° C. After thorough mixing, it was allowed to stand at room temperature for at least five hours, at which time the supernatant liquid was carefully siphoned off, concentrated, under 25 inches of vacuum at a temperature between 51.5 and 60° C. to a thick, syrupy mass usually testing from 35 to 45 per cent total solids and forming a gel at 20° C. This is equivalent to a concentration ratio of approximately thirty to one. Care was exercised to avoid bubbling of any air through the extract during the concentrating process and to avoid metal contamination at any time. The thick syrupy residue was immediately transferred to a tightly stoppered flask and stored in the dark at 4.5° C.

The acetone, ethyl alcohol, ethyl ether, and hexane extracts were prepared in a similar manner. A mixture of twenty per cent oat flour (or soya flour) in the solvent at 20° C. was vigorously shaken in a glass container for five minutes at intervals during one hour. After standing overnight, the supernatant liquid was carefully siphoned off and concentrated in a vacuum flask distillation apparatus, avoiding any metal contamination or any bubbling of air through the concentrate. The concentration was continued until the solvent had been removed. The thick, oily residues which remained were immediately transferred to tightly stoppered flasks and stored at 4.5° C. Although no attempt was made to determine the keeping quality of the extracts they did not appear to lose their antioxygenic properties during two

months storage, provided they were properly prepared, particularly with regard to undue contact with air.

When studying antioxidants in dry milk fat their influence was measured by means of the peroxide number determined by the procedure of Greenbank and Holm (6). The antioxygenic influence of a substance is always measured by the time necessary to produce oxidation of the fat. Frequently this influence is referred to as the "protective factor" which is the ratio of the induction period of the treated sample to the induction period of the untreated sample. For example, if the treated sample had an induction period of nine days while the untreated sample had an induction period of six days, the "protective factor" would be 9/6 or one and one-half. The question immediately arises as to the accuracy of each of the figures nine and six; in other words, how close to six days would be the induction period of replicate samples of the untreated fat all being exposed to the same experimental conditions. This was determined for the particular experimental conditions employed in this investigation by the following experiment.

Test tubes from one shipment, to insure uniform composition and size, were prepared by boiling in alkali solution, thoroughly rinsing, soaking overnight in cleaning solution, thoroughly rinsing with clean tap water and then with distilled water, and finally rinsed, with alcohol and ether before drying in a hot oven. New cork stoppers were thoroughly washed and rinsed and then boiled thirty minutes in distilled water, and air-dried. The same volume of dry fresh milk fat was carefully introduced to each tube to avoid incorporating any air or splashing any fat on the sides of the tube. In this manner the surface area exposed was the same in all tubes. The tubes were stoppered, and set upright in one basket and always handled to prevent any change in the amount of surface exposed. The basket was placed in a small incubator which could accommodate four such baskets and was maintained at 60° C. $\pm$ 1° C. Thus, all tubes were as near identical as the conditions of the experiment would permit. When a tube was removed, it was vigorously shaken to mix its contents and then the peroxide number determined. This tube was then discarded and not returned to the incubator. At critical points several tubes from different positions in the basket were removed and tested within the same half hour to determine whether the induction period was the same for all the tubes.

At the same time, the same fat was placed in several carefully cleaned and dried 50 ml. centrifuge tubes. These tubes were set in baskets which occupied different positions in the incubator and the tubes were permitted to lean at varying angles instead of standing upright, thereby permitting variations in the amount of surface exposed. Portions of the fat in these tubes were removed by a carefully cleaned and dried pipette for determining the peroxide number, and then the tubes returned to the incubator. This possibly increased the exposed surface area by the thin film of fat

adhering to the side of the tube as the fat was removed. Furthermore, these tubes were not stoppered but the entire basket was covered with aluminum foil to prevent the possibility of dust falling into the tubes.

The results appear in table 1 and indicate that the induction period

TABLE 1

The reliability of the induction period as indicated by the peroxide number under these experimental conditions

Days in dark at 60° C.	Peroxide numbers					
	Samples stored in stoppered test tubes					
	1	2	3	4	5	6
0	0.0					
3	0.0					
5	0.0	0.28	0.0			
7	1.20	1.38	0.28			
9	1.17	0.90	0.45	0.09	1.26	0.0
11	1.14	1.14	0.44	0.70	8.80	4.84
13	1.12	12.90	6.45			
15	1.68	16.38				
	Samples stored in 50 ml. centrifuge tubes					
0	0.0	0.0	0.0	0.0	0.0	
3	0.0	0.0	0.0	0.0	0.0	
5	0.19	0.19	0.75	0.85	1.22	
7	0.18	0.18	1.01	1.38	1.84	
9	0.45	0.99	2.25	4.32	7.38	
11	0.86	1.14	7.22	9.42		
13	1.55	1.81				
15	2.10	2.10				

may vary as much as four days between identical tubes of fat under the conditions employed. This difference was easily observed in the fading of the natural color of the fat in the various tubes. Thus, a difference of four days between the induction period of a treated sample and that of an untreated sample would be significant only provided it persisted in several repetitions of the experiment. This limit of error obviously applies only to the experimental conditions employed and would be different when any change, such as incubation temperature, in the experimental conditions was made.

The explanation for this variation in the induction period of identical samples of milk fat is not available, but the most plausible cause would be slight variations in incubation temperature. It was known that the temperature of the fat did not vary as much as one degree and that such a variation should not significantly influence the length of the induction period. There is also the possibility that the complex chain reaction of fat oxidation is not always initiated at the same time in every identical tube of the same fat. Obviously, the autocatalytic nature of the reaction might cause the

reaction to progress slightly faster in one tube than in another when the reaction was once started, and thus change the time necessary to develop a given peroxide number.

RESULTS

The required amount of the fraction of antioxidant was weighed into 50 ml. centrifuge tubes and sufficient dry fat was added to make a total of 35 grams of mixture. The mixture was then incubated in the dark at 60° C. and the peroxide number determined every other day, until a peroxide number of 20 was reached.

Typical results using oat flour fractions are shown in figures 1 and 2 and table 2. They indicate very slight antioxidative powers in all of the

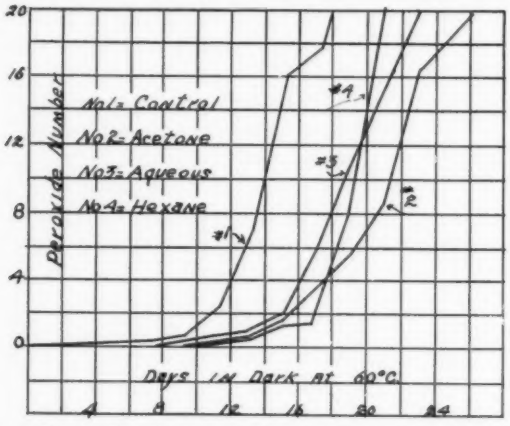

FIG. 1. Antioxygenic influence of 0.1% oat flour extracts in milk fat.

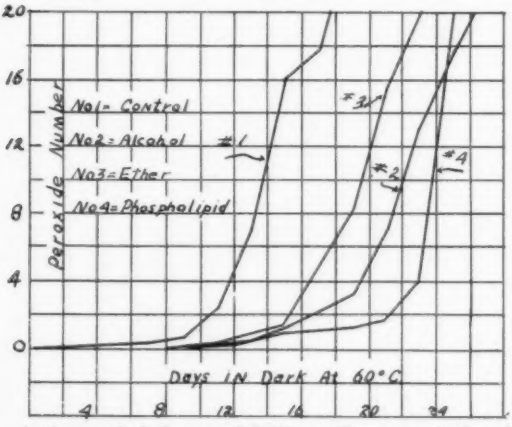

FIG. 2. Antioxygenic influence of 0.1% oat flour extracts in milk fat.

TABLE 2

Antioxygenic influence of extracts of oat flour in dry, milk fat

Days in dark at 60° C.	Control	Peroxide number											
		Acetone extract		Alcohol extract		Ether extract		Aqueous extract		Hexane extract		Phospholipid fraction	
		0.05%	0.10%	0.05%	0.10%	0.05%	0.10%	0.05%	0.10%	0.05%	0.10%	0.05%	0.10%
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.7	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.5	0.3	0.2	0.1
11	2.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	1.2	0.6	0.5	0.3
13	7.0	0.7	0.6	0.5	0.5	0.7	0.7	0.5	0.5	1.7	0.8	1.0	0.8
15	16.2	1.8	1.8	1.4	1.1	1.6	1.5	1.2	1.2	6.5	1.9	2.3	1.0
17	17.9	1.6	1.4	1.6	1.0	6.0	1.4	1.3	1.4	11.0	5.8	4.7	0.8
19	27.3	5.6	4.8	6.2	3.1	25.2	8.2	10.8	8.0	17.3	10.6	7.9	1.3
21		8.8	8.6	10.0	7.0		15.5	24.5	22.5	21.5	15.2	12.3	1.7
23		15.0	16.5	16.3	13.0		38.8				21.8	17.5	4.0
25		18.9	19.6	20.3	16.7							22.2	28.4
27					24.8								

extracts. Lower concentrations (five hundredths per cent) of the extracts were used with similar results, the antioxygenic power being only slightly less than for one-tenth per cent concentrations in each case. The alcohol extract and the phospholipids exhibited the greatest power, but some of the other extracts seemed to exhibit quite similar antioxygenic powers although they did not always appear in the same order in repeated experiments. The increased induction period due to some of the extracts is so close to the limit of error of the method that its significance might seem questionable. However, in repeated experiments these extracts always exhibit this same small protective power, and therefore it is considered to be significant.

The typical results using soya bean flour extracts appear in figures 3 and 4 and table 3. Here also the low (five hundredths per cent) concen-

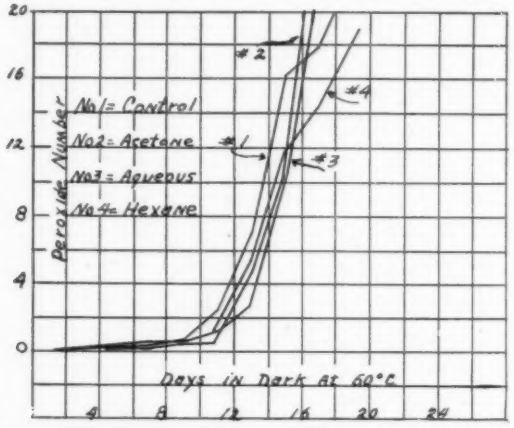

FIG. 3. Antioxygenic influence of 0.1% soya flour in milk fat.

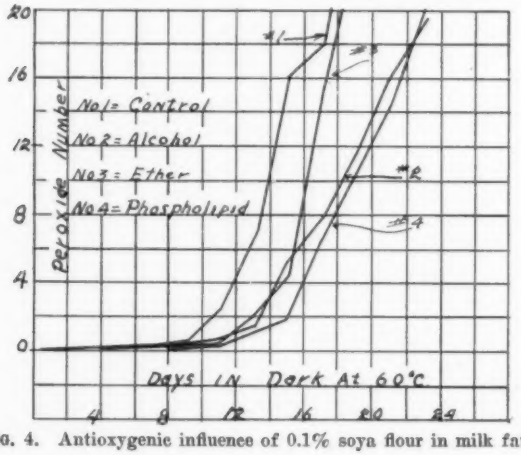

FIG. 4. Antioxygenic influence of 0.1% soya flour in milk fat.

TABLE 3

Antioxygenic influence of extracts of soya flour in dry, milk fat

Days in dark at 60° C.	Control	Peroxide number											
		Acetone extract		Alcohol extract		Ether extract		Aqueous extract		Hexane extract		Phospholipid fraction	
		0.05%	0.10%	0.05%	0.10%	0.05%	0.10%	0.05%	0.10%	0.05%	0.10%	0.05%	0.10%
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	0.4	0.5	0.5	0.3	0.3	0.0	0.0	0.3	0.3	0.4	0.5	0.0	0.0
9	0.7	0.6	0.5	0.6	0.5	0.4	0.3	0.5	0.4	0.5	0.4	0.2	0.1
11	2.4	1.0	1.2	0.7	0.7	0.6	0.4	0.9	0.5	0.9	1.1	0.5	0.3
13	7.0	3.9	2.7	1.4	1.4	2.0	2.0	12.4	4.6	4.3	5.4	1.6	1.5
15	16.2	14.6	11.4	8.8	5.2	8.4	4.4	20.2	10.2	9.8	10.8	2.6	1.9
17	17.9	32.0	26.3	12.3	7.9	25.7	15.2		29.6	12.9	14.2	8.4	6.4
19	27.3			22.9	11.7		30.4			18.3	19.1	12.6	10.3
21					16.0							20.8	14.5
23					19.1								20.0

trations exhibited practically the same protective power as did the one-tenth per cent concentrations of each fraction.

The alcohol extract and the phospholipid fraction exhibited greater antioxygenic influence than any of the other extracts of soya bean flour, while some of the other extracts appeared to exhibit insignificant protective powers. It was also observed that the oat flour fractions were always more effective than the soya bean flour extracts. These results are contradictory to those of Musher (15, 16) who found soya bean flour to be more effective than oat flour when used in lard. Further investigation is necessary to establish an explanation for this apparent disagreement in results, although it may be just another example of the well known fact (5, 17) that an antioxidant for one system may have little effect on another system.

The main object of these experiments was to determine the relative antioxygenic influence of these fractions with the hope of concentrating the active substance. Consequently, no attempt was made to establish the composition of the various extracts and fractions. However, some correlation between composition and effectiveness appears to be indicated.

The phospholipid fraction would be expected to exhibit definite antioxygenic properties since it contains cephalin which is known to be an antioxidant for other fats (18). It also contains lecithin which may be also an antioxidant for this particular system.

The slight effectiveness of some of the extracts, might possibly be due to their small phospholipid content. It is highly probable that they contain phospholipids because the complete removal of phospholipids from fat is notably difficult. The solubility of both lecithin and cephalin in solvents such as ether and alcohol is known (13) to be increased by the presence of moisture or fat. Since fat was present in all of these extracts except possibly the aqueous, the presence of at least traces of phospholipid in some seems inevitable. Whether there is sufficient to account for the antioxygenic properties remains for further investigation. Since many gums are soluble in alcohol and water, and since some gums possess antioxygenic properties, the effectiveness of these extracts may be due to the presence of certain gums.

Therefore from these preliminary experiments, it would seem that there may be more than one active substance in either oat or soya bean flour.

SUMMARY

An attempt was made to determine the active fraction of two antioxidants, oat flour and soya bean flour, with only partial success. The phospholipid fraction and an alcohol extract exhibited greater antioxygenic properties in dry fresh milk fat than did either the aqueous, acetone, ether or hexane extracts. In every case the oat flour extracts appeared to have greater protective powers in dry fresh milk fat than the corresponding extract of soya bean flour.

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THE RELATION OF MASTITIS TO THE LEVEL OF ASCORBIC ACID AND CERTAIN OTHER CONSTITUENTS IN MILK*

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It is well established that mastitis results in changes in the composition of milk, the extent of the alteration being correlated to a considerable degree with the severity of the infection.

Dahlberg, *et al.* (1) reported that only slight differences occurred in the chemical composition of milk from infected as compared to non-infected cows as long as the milk remained normal in appearance, but when the infection became acute the milk composition was grossly affected. Among the changes noted were a decrease in lactose, specific gravity, solids-not-fat, and curd tension, and an increase in chlorides and albumin. However, the changes in composition were no greater than are found between two herds of the same breed. Jones and Little (2) demonstrated that the proteins of the whey fraction in milk are increased as a result of mastitis, the increase being largely due to an increase in the globulin fraction. They suggested that this was due to increased permeability of the blood capillaries to blood proteins because of the inflammation. Of considerable practical importance is the finding of Foot and Shattock (3) and Rowland and Zein-El-Dine (4) that mastitis results in a low percentage of solids-not-fat in milk.

Von Wendt (5) reported that the ascorbic acid content of milk depends upon the health of the udder. He states that if a quarter of the udder becomes infected, the ascorbic acid content of milk from this quarter quickly declines and may fall nearly to zero, and that if all four quarters are infected, the mixed milk may be almost devoid of ascorbic acid. In view of the importance of ascorbic acid in nutrition and also of the need for diagnostic tests for the detection of mastitis, this finding would have considerable significance if fully substantiated.

The present paper includes the results of a study that was conducted in order to determine the relationship between infection in individual quarters of cows in the University herd and the level of ascorbic acid and chlorides in the milk.

EXPERIMENTAL

The cows used for this study were selected from the University of Missouri dairy herd on the basis of previous health records as shown by

Received for publication August 1, 1940.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal series, No. 688.

monthly bacteriological and chemical examination of individual quarter samples of the foremilk. Of the cows sampled, 8 were free from mastitis in all quarters and 11 had one or more infected quarters. However, the milk from all quarters was normal in physical character. The samples were drawn at the morning milking, one sample being taken aseptically for bacteriological examination, and the other being drawn in an amber glass container for the determination of chlorides and ascorbic acid.

Ascorbic acid was determined by titration with sodium 2–6 dichlorobenzenone indophenol (6, 7), in a trichloroacetic-metaphosphoric acid filtrate. In order to inhibit oxidation of the ascorbic acid before analysis, a series of 50 cc. volumetric flasks were prepared before drawing the samples by placing 20 cc. of a solution containing 13.5 per cent of trichloroacetic acid and 4 per cent of metaphosphoric acid in each flask. Immediately after sampling, 20 cc. of milk were pipetted into the acid solution, with agitation; the flasks were immediately taken to the laboratory, filled to the mark with more of the precipitating solution, and filtered. Fifteen cc. aliquots of the protein free solution were then titrated for their ascorbic acid content with indophenol that had been freshly standardized against a pure ascorbic acid solution. A 10 cc. microburette, graduated in 0.02 cc. divisions was used. In checking upon the efficiency of this procedure, it was found that any losses of ascorbic acid occurring between the time of sampling and titration were too small to be measurable by the titration procedure used.

The following determinations were also made on the milk: (a) Chlorides by the digestion method of Davies with the results expressed as per cent sodium chloride; (b) catalase test; (c) number of leucocytes by the Breed smear technique; (d) plate count of bacteria using beef infusion agar and (e) the type of bacteria by microscopic examination of the incubated milk.

TABLE 1

The average level of chlorides and ascorbic acid in negative, doubtful and positive quarters

	No. of samples	Chlorides as NaCl (mg. per cent)	Ascorbic acid (mg. per L.)
Negative	48	90.9	20.4
Doubtful	9	106.9	19.1
Positive	33	124.7	18.4
All samples	90	104.9	19.5

The presence or absence of infection in the individual quarter samples was determined on the basis of the catalase test, leucocyte count, and number and type of bacteria present. Of the 90 quarter samples analyzed 48 were definitely negative; 9 were doubtful, and 33 were positive for mastitis. Table 1 shows the average level of chlorides and ascorbic acid in the 3 classifications indicated. While there is a trend toward a higher level of chlorides and a lower level of ascorbic acid in milk from positive quarters, there is

a quite narrow margin of difference in the ascorbic acid values. In fact, the average ascorbic acid differences obtained are no greater than the range of values sometimes encountered in the individual quarter samples from a mastitis free udder.

The relationship between chlorides and ascorbic acid in the positive and negative quarters is shown in the scatter diagrams pictured in figures 1 and 2. While there is a great deal of variability in the values shown, the line

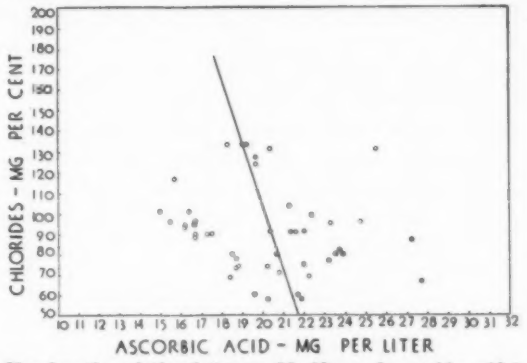

FIG. 1. Showing the relation between chlorides and ascorbic acid in mastitis negative quarters.

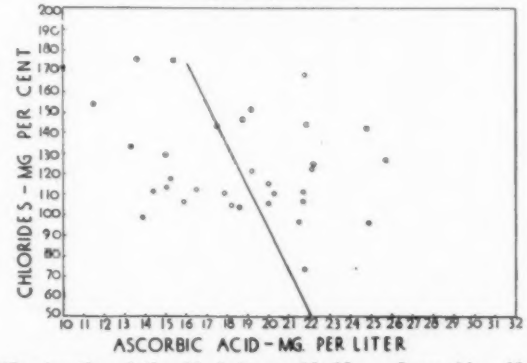

FIG. 2. Showing the relationship between chlorides and ascorbic acid in mastitis positive quarters.

of average relationship, as computed by the method of least squares shows in both series a trend toward a decrease in ascorbic acid concentration as the level of chlorides increases. The coefficient of correlation between ascorbic acid and chlorides in the negative quarters is -0.1057; probable error 0.1427, a value too small to be statistically significant. In the positive quarters the correlation is -0.3228; probable error 0.1559. It thus appears that, though small, there is an inverse relationship between the level of chlorides

and ascorbic acid in the infected quarters of the udder. The data considered above include only negative cows and cows with relatively mild cases of mastitis, all of which produced milk that was normal in appearance. However, from analyses that were secured from a few cows in advanced stages of mastitis it appears that the inverse relationship between chlorides and ascorbic acid becomes much more evident as the infection progresses.

Table 2 contains detailed analyses of samples drawn from 4 negative cows and 3 cows having 1 or more quarters seriously infected.

TABLE 2

Detailed analyses of individual quarter samples from mastitis negative and positive cows

Cow No.	Chlorides as NaCl (mg. per cent)	Ascorbic acid (mg. per L.)	Catalase (cc. gas)	Leucocytes (No. per cc.)	Number of bacteria per cc.	Type of bacteria	Diagnosis for mastitis
747	90.37	17.3	0.2	0	700	Staph.	−
	90.37	16.7	0.2	99,400	700	Staph.	−
	88.60	16.7	0.2	0	400	Staph.	−
	90.37	17.5	0.2	0	200	Staph.	−
753	79.94	20.7	0.2	198,800	200	Staph.	−
	79.94	18.5	0.3	149,100	300	Strep.	−
	77.97	18.7	0.8	149,100	900	Staph.	−
	74.42	20.2	0.4	248,500	200	Staph.	−
900	60.25	19.6	0.4	23,950	100	Strep.	−
	58.48	20.2	0.3	99,400	100	Staph.	−
	60.25	21.8	0.4	0	200	Staph.	−
	58.48	22.0	0.5	49,700	200	Staph.	−
889	69.11	18.4	0.9	49,700	100	Micro.	−
	74.42	18.9	0.9	0	100	Micro.	−
	72.65	18.7	1.2	347,900	200	Staph.	−
	70.88	20.8	0.8	23,950	100	Staph.	−
427	113.40	14.97	2.5	1,391,600	12,000	Strep. Staph.	+
	129.35	14.93	2.4	4,125,100	3,000	Staph.	+
	95.69	15.90	0.6	497,000	800	Micro.	+
	109.86	14.41	2.1	1,988,000	2,000	Strep. Staph.	+
435	175.43	13.6	5.6	6,659,800	5,600	Strep. Staph.	+
	95.69	21.5	1.4	670,950	7,100	Strep. Staph.	+
	175.43	15.4	4.6	11,530,400	15,300	Strep. Staph.	+
	73.28	21.8	0.6	49,700	7,000	Strep. Staph.	+
100	97.46	17.2	1.2	650,000	3,200	Strep. Staph.	+
	99.23	16.4	0.8	220,000	2,100	Staph.	+
	184.28	11.4	5.4	2,500,000	22,000	Strep.	+
	133.01	14.6	0.9	45,000	1,200	Micro.	+

These data, though limited, indicate that in advanced cases of mastitis the ascorbic acid content may be reduced to one-half to two-thirds of its normal level. At the same time the chlorides are markedly increased.

This brings up the question as to whether the depression in ascorbic acid content in milk from cows that are infected with mastitis is due to a direct effect of the bacteria in oxidizing the ascorbic acid in the milk, or whether it is an indirect effect, caused by the changes in properties generally believed to take place in the milk secreting tissue during periods of infection.

In order to test the first possibility a sample of milk was sterilized in the autoclave, following which a small amount of crystalline ascorbic acid was added. The milk was then divided into four equal portions, and a culture of a long chain *Streptococcus* isolated from mastitis milk was introduced into two of them, the other two serving as controls.

The samples were allowed to stand at room temperature, but protected from strong light for 24 hours, during which time ascorbic acid determinations were made periodically. The results are shown in figure 3. From

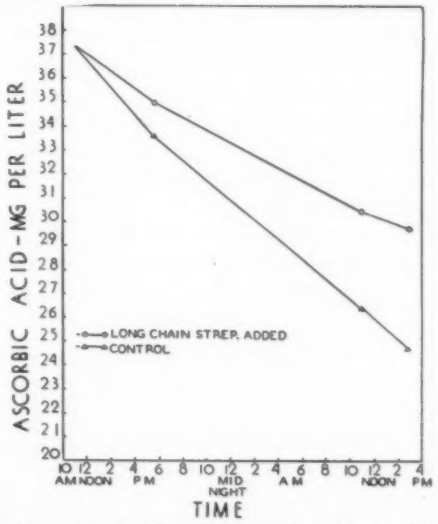

FIG. 3. Effect of long chain *Streptococcus* on the rate of oxidation of ascorbic acid in milk.

the curves shown it is apparent that the *Streptococcus* organism actually retards the rate of oxidation of ascorbic acid. Thus it is hardly possible that bacteria could be directly responsible for the lowered level of ascorbic acid in mastitis milk.

In order to determine whether or not the lowered ascorbic acid content of mastitis milk was due to a higher level of ascorbic acid oxidase in the mastitis

milk, the ascorbic acid oxidation curve was followed in samples of milk drawn from positive and negative quarters. The rate of oxidation was slightly less in the positive sample, probably because of the higher bacterial count of this sample. Although the bacteria present are a complicating factor in this experiment it appears that there is little if any difference in the ascorbic acid oxidase content of the two types of milk.

The reports of several investigators have shown that the experimental alteration of either the osmotic or hydrostatic pressure in the mammary gland results in marked changes in the composition of milk. Garrison and Turner (9) reported on the effect on milk secretion of the injection into the udder of water and solutions of salt, lactose, milk, oil and oxygen. They point out the close similarity in composition between milk altered by such treatment and milk drawn from quarters that are infected with mastitis.

With this relationship in mind it was believed that further light might be thrown on the mechanism governing the reduction of ascorbic acid in mastitis milk by producing a sterile inflammation in the udder through injection of a hypertonic salt solution. Cow 677, selected for the injections, had a mastitis free history, and analyses made prior to this experiment showed practically identical values for chlorides and ascorbic acid in milk from all four quarters of her udder.

Immediately following the afternoon milking 1000 cc. of a solution containing 3.5 per cent lactose and 0.14 per cent sodium chloride was injected into each right quarter of the udder, and samples were drawn for analysis each afternoon during the next 7 days.

The changes in the level of chlorides and ascorbic acid in the injected as compared to the uninjected half of the udder are shown in figure 4. The

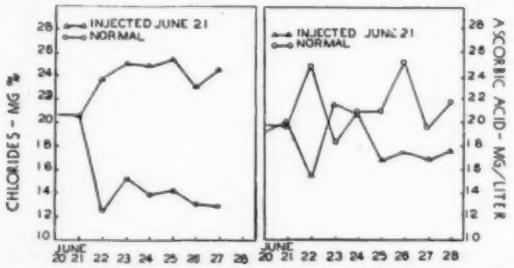

FIG. 4. Changes in the ascorbic acid and chloride content of milk following injection of a hypertonic sodium chloride-lactose solution.

chlorides in the injected quarters rose sharply on the day following the injection, while the chlorides in the uninjected half declined sharply during the same period. Although not nearly so uniform, the ascorbic acid levels showed a trend in the reverse direction from that of the chlorides. On the first day the ascorbic acid level dropped abruptly in the injected half and

rose sharply in the normal half of the udder. This was followed on the second and third days by a rise in ascorbic acid in the injected quarters, accompanied by a decline in the normal quarters. Subsequent to the third day, the ascorbic acid level dropped to a uniformly low level. Samples analyzed on the 16th day after injection showed that all quarters had returned to normal by this time. Running parallel to the changes noted above there was an increase in the catalase content and an extremely high leucocyte count in the injected quarters. The plate count for bacteria showed no significant change throughout the course of the experiment.

DISCUSSION

From the results obtained on milk from cows in early stages of mastitis it appears that such infections tend to affect adversely the level of ascorbic acid in the milk. In the cases studied, the decrease in ascorbic acid in the mastitis positive as compared to the negative quarters was of the order of 10 per cent whereas the chlorides increased approximately 35 per cent. In more advanced cases of mastitis the ascorbic acid content of the milk was reduced as much as 30 to 50 per cent while the chlorides increased as much as 100 per cent. However, no cases were encountered in which the depression in ascorbic acid was as severe as that reported by VonWendt (5).

If, as is generally believed, the symptoms of mastitis are produced as the result of bacterial infections in the mammary gland, it is of interest to learn the actual role of the infectious organism in producing the effect noted. The results pictured in figure 3 show that when long chain streptococci of the type generally associated with mastitis are present they actually retard the oxidation of ascorbic acid. That this is true is not surprising inasmuch as the bacteria probably consume oxygen that otherwise would be available for the oxidation of ascorbic acid.

This observation is supported further by the recent study of Esselen (10) who reported that most of the micro-organisms investigated by him significantly retarded the oxidation of ascorbic acid, especially when cultured in media containing readily fermentable carbohydrate.

The decreased level of ascorbic acid in milk from infected as compared to normal quarters of the udder might be thought to be due to a larger amount of ascorbic acid oxidase in the mastitis milk, which in turn would bring about a faster rate of disappearance of the vitamin. That this is not the case, however, is indicated by experiments reported in the present paper.

Von Wendt (5) suggested that the ascorbic acid in milk is synthesized in the secretory tissue of the mammary gland from some precursor in the blood, probably sorbose, instead of being obtained directly from the ascorbic acid in the blood. A decrease in ascorbic acid in the milk when the quarter is infected is then explained by a failure of the pathological tissue to synthesize ascorbic acid from the blood precursor.

It seems probable that the diminution of ascorbic acid is caused indirectly by the injurious effect of the bacteria in altering the character of the secretory epithelium of the mammary gland. The fact is well established that in severe cases of infection the permeability of the milk secreting cells is seriously altered and the milk becomes more like blood serum in character. There is a reduction in lactose and an increase in chlorides, in addition to other changes previously referred to. Garrison and Turner (9) suggest that in such cases "*the increased permeability of the cells to chlorides is the cause* and the low lactose secretion the result of the cells effort to maintain osmotic equilibrium." Rasmussen, *et al.* (11) demonstrated that the level of ascorbic acid in milk can be temporarily increased by the intravenous injection of crystalline ascorbic acid, a finding that is in harmony with the assumption that the ascorbic acid of the milk has its origin in the preformed ascorbic acid of the blood. The tendency for the ascorbic acid of the milk to decrease as the chlorides increase, both in the case of mastitis infection and following the perfusion of the mammary duct system with a hypertonic salt solution indicates that the transfer of ascorbic acid from the blood stream to the milk is controlled, at least in part, by the complex equilibrium system suggested above.

Although there have been some reports to the contrary, it appears to be firmly established that the ascorbic acid content of the diet has little if any effect upon the ascorbic acid content of cow's milk (12, 13, 14, 15, 16). This rather surprising fact is explained by the report of Knight, *et al.* (17) that ingested ascorbic acid is rapidly destroyed in the rumen. When we consider further the fact that ascorbic acid can be readily synthesized by cattle (Thurston, Palmer and Eckles (18)), it appears that the ascorbic acid in cows milk is provided to a large extent by an endogenous source or sources. Thus the amount of ascorbic acid secreted in milk would be due to two major factors:

a. The rate of synthesis and discharge of ascorbic acid, which in turn would control the level of ascorbic acid in the blood, and

b. The permeability of the milk secreting cells to ascorbic acid in relation to the osmotic equilibrium existing between the blood and the milk. While the first factor may be assumed to be relatively constant, the present work indicates that upsets in the second factor due to mastitis infections or other causes may result in considerable variations in the level of ascorbic acid in milk.

Von Wendt (5) recommended the measurement of ascorbic acid as a diagnostic measure for the detection of mastitis. However, the present study indicates that the reduction in ascorbic acid as a result of infection is not specific enough, especially during the early stages, to offer any advantages over the common methods of diagnosis.

SUMMARY

As the result of a study of the ascorbic acid content of milk drawn from mastitis positive as compared to mastitis negative quarters, it is concluded that udder infections reduce the amount of ascorbic acid in the milk, the reduction being correlated in general with the severity of the infection. In early stages of mastitis the ascorbic acid of the milk was reduced about 10 per cent and in advanced cases it was reduced by as much as 30 to 50 per cent. It was shown that there is a trend toward an inverse relationship between ascorbic acid and chlorides in mastitis milk and also in the milk produced following perfusion of the mammary duct system with a hypertonic solution.

Long chain streptococci isolated from mastitis milk were shown to actually retard the rate of oxidation of ascorbic acid *in vitro*. A higher ascorbic acid oxidase content in mastitis milk was also ruled out as a factor causing the reduction in ascorbic acid.

The theory is advanced that the effect of mastitis upon the level of ascorbic acid in milk is exerted indirectly by producing a change in the selective permeability of the milk secreting cells in relation to the osmotic equilibrium existing between blood and milk.

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PRELIMINARY OBSERVATIONS ON CHEMICAL CHANGES OF RUMEN INGESTA WITH AND WITHOUT UREA[1]

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INTRODUCTION

Previous *in vitro* work at this station (2) on the utilization of inorganic nitrogen by ruminants has indicated the necessity of *in vivo* investigations on this problem. To obtain the necessary conditions, the observations herein reported were made using a heifer with a rumen fistula. To date little work on rumen chemistry has been reported. The work herein reported is being carried on as a part of the study of the utilization by ruminants of inorganic nitrogen as a source of protein. To interpret the changes in the chemical composition of rumen ingesta brought about by the addition to the ration of inorganic nitrogen, it was first essential to have a knowledge of the normal conditions and changes taking place in the rumen.

EXPERIMENTAL

By way of the fistula, aliquots of rumen material were removed periodically for analysis throughout the day beginning approximately one hour after the animal was fed. Care was taken in removing an aliquot from the rumen since considerable variation was found in the chemical composition of the contents obtained from various parts of the paunch. To secure a representative sample, all parts of the paunch (rumen) were sampled to make up the aliquot. The average weight per aliquot was approximately one kilogram of material. Analyses made on the rumen ingesta and the animal's ration included pH, dry matter, ammonia nitrogen, total nitrogen, non-protein nitrogen, (phosphotungstic acid non-precepitable nitrogen) fiber, ether extract, and urea nitrogen. All analyses are reported on the dry basis.

Composition of rumen contents on the basal ration. The basal ration which the fistula heifer received per day consisted of 6 pounds of timothy hay, 6 pounds of a grain mixture (ground corn ½ plus ground oats ½) and 18 pounds of corn silage. The data obtained are shown in table 1.

Dry matter was determined by drying 400 grams of the material removed

Received for publication August 2, 1940.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

We are very grateful to Dr. W. Wisnicky for the successful rumen fistula produced in the heifer used in these experiments and to E. I. Du Pont de Nemours and Company, whose financial assistance aided greatly in the execution of this study.

[2] Wisconsin Alumni Research Foundation Scholar.

from the rumen in a steam oven for 24 hours at 98° C. Fiber and ether extract were then run on the dry material using the A. O. A. C. methods. Ammonia nitrogen was determined by placing 20 grams of the undried rumen ingesta into a Kjeldahl flash to which 300 cc. of water and 5 grams of magnesium oxide were added. The ammonia was then distilled into standard acid. Foaming was prevented by adding liquid paraffin. The non-protein nitrogen was determined by taking 20 grams of the undried rumen contents, adding 10 cc. of a 10 per cent solution of phosphotungstic acid plus 5 cc. of 1.2 N sulfuric acid and making the volume to 100 cc. This was then shaken and allowed to stand for 30 minutes after which it was centrifuged and 50 cc. of the supernatant liquid transferred to a Kjeldahl flask and total nitrogen determined by the Kjeldahl method. Total nitrogen on the rumen material was also determined by the Kjeldahl method.

TABLE 1

Rumen content analysis—basal ration

Time after feeding	Crude protein	Non-protein nitrogen	Ammonia nitrogen	Fiber	Ether extract	pH
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	
Ration	8.45	0.17	0.044	21.7	3.0	.....
1 hour	10.0	0.250	0.065	.....	2.80	.....
3 hours	11.5	0.090	0.016	34.0	3.10	6.30
5 hours	11.8	0.085	0.014	35.2	2.90	6.65
9 hours	10.8	0.091	0.019	36.0	2.70	7.15
12 hours	10.4	0.080	0.021	37.4	2.67	7.20
15 hours	10.5	.....	0.046	38.8	2.30	7.40
18 hours	10.4	.....	0.040	38.1	2.50	7.30

From the values obtained it was observed that the moisture content of the ingesta increases slightly with time after feeding. This is explained on the assumption that part of the rumen content is passing out and that moisture is being added through salivation and drinking of water. It was also observed that the rumen was only partially emptied between feedings.

The ammonia nitrogen constituted a very small part of the total nitrogen in the paunch when the cow was on the basal ration, being from 1 to 3 per cent of the total nitrogen present. Further, the ammonia nitrogen which was introduced in the feed decreased to a very low level in 4 to 6 hours after feeding. Non-protein nitrogen was run in order to estimate what percentage of the total nitrogen existed in a non-protein form. Using phosphotungstic acid it was found that about 4 to 6 per cent of the total nitrogen in the rumen material appeared in the non-protein nitrogen fraction and remained fairly constant throughout the day.

Considering the total nitrogen, it can be seen that there was a decidedly higher value for the rumen material over that found in the basal ration, and further, the total nitrogen increased slightly for several hours after

feeding. At the same time it is seen that the fiber likewise was higher in the rumen material than in the ration immediately after feeding and increased for several hours thereafter. The pH values which we observed were in accord with the results reported by other workers (1), being lowest for several hours after feeding and becoming more alkaline as the time for the next feeding approached. The percentage of ether extract found, paralleled that found in the ration and was a minor constituent of the rumen ingesta.

Effect of adding urea to the basal ration. Having established the composition of the rumen material when the basal ration was fed, the composition of the ingesta with added urea was next studied. The urea was added at a 1 per cent level of the dry matter of the ration. The results secured are recorded in table 2.

TABLE 2

Rumen content analysis—basal ration plus urea

Time after feeding	Crude protein	Non-protein nitrogen	Ammonia nitrogen	Urea plus ammonia nitrogen	Fiber	Ether extract	pH
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Ration + urea	11.3	0.985	0.075	.....	22.15	3.87	.....
1 hour	12.0	0.344	0.222	0.214	.....	.....	6.60
2 hours	11.4	0.289	0.181	0.187	32.9	3.57	6.75
3 hours	11.7	0.208	0.122	0.118	.....	.....	6.50
4 hours	11.8	0.193	0.072	.....	.....	.....	6.50
5 hours	11.5	0.106	0.030	.....	33.8	3.23	6.70
9 hours	12.1	0.087	0.023	.....	33.1	2.85	6.85
12 hours	11.3	0.103	0.036	.....	35.2	2.42	7.40
15 hours	11.3	0.105	0.035	.....	34.7	.....	7.55
18 hours	11.6	0.118	0.047	.....	36.4	2.65	.....

In comparing tables 1 and 2 it is seen that the values for ether extract, dry matter, and fiber are unchanged. It has been suggested that the addition of urea to the ration might enhance the solution of cellulose (fiber). There is no clear-cut evidence from the available data that a greater solution of fiber occurs in the rumen when urea is added to the basal ration.

Considering the nitrogen picture it is seen from table 2 that within one hour after feeding, the urea had completely disappeared, having been hydrolyzed to ammonia nitrogen, or converted to protein. Urea nitrogen was determined by weighing out 20 grams of rumen contents into a Kjeldahl flask and adding 2 cc. of chloroform plus 2 cc. of toluene to stop bacterial action. The volume was made up to approximately 100 cc. and the pH adjusted to 7. Ten cc. of a 1 per cent urease solution were then added and allowed to act for one hour, after which time the ammonia produced from the hydrolyzed urea was determined as in the ammonia determination. Any increase in the ammonia nitrogen after urease treatment over the ammonia nitrogen was considered to be urea nitrogen.

From table 2 it can also be seen that the per cent of ammonia nitrogen found on the basal plus urea ration was initially high as would be expected due to the hydrolysis of the added urea to ammonia. After 4 to 6 hours it had decreased to approximately the same level as the basal ammonia nitrogen indicated in table 1, and, in the same time.

This practically complete disappearance of ammonia nitrogen is offered as evidence of protein synthesis since its disappearance could not be accounted for by its selective removal from the rest of the rumen ingesta under the acid conditions which prevailed. The increase in non-protein nitrogen over that found in the basal ration can also be accounted for by the added urea. The same drop in the non-protein nitrogen at the end of 4 to 6 hours occurred as was observed in the case of the ammonia nitrogen.

The initial total nitrogen was also higher on the urea plus basal ration because of the increased ammonia nitrogen present. The analyses of samples taken several hours after the initial run showed only a slight increase over the basal ration. However, there was some evidence of an increase in protein in the rumen ingesta by growth of the microorganisms using the added urea.

Effect of urea added to a low protein basal ration. Failure to demonstrate a larger protein increase than 10 per cent by the addition of urea to the basal ration led to the use of a new basal ration lower in total nitrogen. On a lower protein basal ration any protein synthesis would be more easily detected since the per cent of the newly formed protein would constitute a greater part of the total nitrogen. Several different basal rations low in nitrogen were tried. The basal ration which gave more positive evidence of a protein synthesis on addition of urea contained 15

TABLE 3

Composition of rumen contents on low protein ration and low protein ration plus urea

Date	Time after feeding	Ration	Ammonia nitrogen	Crude protein in rumen content
			per cent	*per cent*
6/14	1 hour	Basal	0.015	9.65
6/14	5 hours	"	0.006	9.85
6/14	8 "	"	0.005	10.10
6/18	3 "	"	.....	9.24
6/19	2 "	"	.....	10.40
6/20	2 "	"	.....	10.60
6/20	8 "	"	.....	8.90
6/21	2 "	"	.....	9.50
6/26	2 "	Basal + 5% urea	0.168	12.70
6/26	8 "	" + 5% "	0.051	12.0
6/27	2 "	" + 5% "	0.150	12.65
6/27	8 "	" + 5% "	0.061	12.65
7/5	2 "	Basal	0.067	9.45
7/5	8 "	"	.....	10.30
7/6	2 "	"	0.070	9.46

pounds of corn silage and 3 pounds of starch. It is realized this basal ration was too low in protein ($N \times 6.25 = 4$ per cent) to be a practical ration, but it was satisfactory for the purposes of this experiment. The urea added constituted 5 per cent of the dry matter of the ration. The results secured are shown in table 3.

From table 3 it can be seen that on both the basal ration and the basal ration plus urea, the ammonia nitrogen was at a low level several hours after feeding, but the protein level of the rumen contents after adding urea was approximately 20 per cent higher. The increase in total nitrogen was due to the formation of protein nitrogen since the filtrate nitrogen always returned to the same low level found on the basal ration.

DISCUSSION

It is readily seen that one of the difficulties which presented itself in the above work was that the rumen ingesta is part of a moving system, some of the contents periodically passing out and water and saliva passing in. However, as can be seen from table 3 the conditions are constant enough from day to day to make duplication of analyses possible. However, care in sampling was essential to insure uniform results. Securing samples of the rumen contents apparently did not alter the normal conditions of the rumen since the samples represented only a small percentage of the total material and since analysis of single samples agreed with analyses where several samples (5–6) were taken during the day. Since it was possible to duplicate the results on the basal ration (table 3) it was felt that any changes produced by the addition of urea to the ration could be detected. Comparison of the results shown in tables 1 and 2 showed no large differences due to the added urea, but there was evidence of increased protein and fiber with a decrease in the nitrogen-free extract. Further experimental work will be necessary before the question of the influence of urea on the solution of fiber in the rumen can be definitely decided. Experiments to clarify this question are in progress.

Evidence herein presented shows that the total nitrogen and fiber are higher in the rumen ingesta immediately after feeding than in the ration and also increased for several hours after feeding. The initially higher total nitrogen and fiber in the rumen contents above that found in the ration is accounted for by the fact that the residual ingesta in the rumen before feeding is higher than the ration fed. Thus, when the cow eats, the mixture of the ration and rumen material gives a final value for total nitrogen and fiber above that of the ration. The steady increase of total nitrogen and fiber after feeding can be explained by the fact that the nitrogen-free extract leaves the rumen first, being broken down by bacteria or bacterial enzymes and passed out of the rumen into the reticulum. Selective removal of the nitrogen-free extract and by-products causes an increase

in the total nitrogen and fiber. Thus the nitrogen-free extract decreases while the total nitrogen and fiber increase.

The data indicate that the urea nitrogen utilization in the rumen must occur within 4 to 6 hours after feeding, since the urea nitrogen and ammonia nitrogen found are negligible after that time (see table 2). Both the practically complete disappearance of ammonia nitrogen in the rumen and the corresponding increase in total nitrogen over the basal ration (tables 2 and 3) are offered as evidences of protein formation from urea in the rumen. All values reported represent averages of several trials. The quantitative results reported here are applicable only to the ration used. Probably the same general trends could be expected on other rations, although the influence of the ration on the bacterial flora will need study.

SUMMARY

1. A comparison of the composition of rumen contents and the ration fed in respect to dry matter, fiber, ether extract, ammonia nitrogen, non-protein nitrogen and total nitrogen has been made.

2. The level of total nitrogen and fiber found in the rumen material is distinctly higher than in the ration fed.

3. The level of total nitrogen (protein nitrogen) and crude fiber found in the rumen content increases slightly in the time between feeding, indicating a more or less selective removal of nitrogen-free extract due to its breakdown by bacteria or bacterial enzymes and its passage out of the rumen.

4. Urea nitrogen or ammonia nitrogen when ingested as 1 to 5 per cent of the dry matter of the ration disappeared from the rumen in 4 to 6 hours after feeding. Evidence for its conversion to protein nitrogen is presented.

5. A definite increase in the percentage of protein nitrogen of the rumen ingesta was produced by adding 5 per cent urea to a low nitrogen basal ration.

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THE RIBOFLAVIN CONTENT OF MILK AS INFLUENCED BY DIET[1]

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In 1938 a project was begun at this station to study the influence of the riboflavin content of the ration upon the vitamin content of milk. In experiments with cows, rations of natural feeds have been used, comparing pasture feeding with a winter ration designed to be low in the vitamin and with a normal winter ration. In experiments with goats, more critical studies have been made by employing the purified diet technique. The riboflavin content of the milk was determined by the method of Hand (3). These studies are here described.

EXPERIMENTS WITH COWS

In the first experiment, two Holstein cows were studied over a period of 163 days, using different rations and involving a total of 120 riboflavin determinations on the milk of each animal. At the outset, determinations were made daily, and sometimes at each milking, for ten days while the cows were on excellent, early season pasture, supplemented with the regular herd concentrate mixture,[2] representing a ration rich in the vitamin.

The cows were then changed to a ration designed to be very low in riboflavin. The daily concentrate allowance consisted of 12 pounds of the following mixture: corn gluten meal, 35 parts; ground wheat, 35 parts; ground oats, 23 parts; molasses, 5 parts; salt, 1 part; bone meal, 1 part. A poor grade of timothy hay was fed ad libitum as roughage. By the method of Hodson and Norris (5) the concentrate mixture was found to contain 0.5 microgram of riboflavin per gram and the hay 2.9 micrograms per gram. Thus the day's ration of each animal contained from 35 to 50 milligrams of the vitamin. The cows were fed this ration for 62 days and then the concentrate mixture was supplemented with 10 per cent of a molasses-yeast byproduct.[3] This supplement increased the daily riboflavin intake by 15 milligrams or 30 to 40 per cent.

Received for publication August 8, 1940.

[1] This study was supported in part by the Cooperative G. L. F. Exchange, Ithaca, N. Y., and by U. S. Industrial Alcohol, New York, N. Y.

[2] The grain mixture was made of linseed oil meal, 200 lbs.; wheat bran, 360 lbs.; hominy feed and corn meal, 340 lbs.; ground oats, 37 lbs.; corn distillers' dried grains, 300 lbs.; cane molasses, 100 lbs.; dicalcium phosphate, 15 lbs.; ground limestone, 5 lbs.; salt, 10 lbs.

[3] The molasses-yeast byproduct is produced by U. S. Industrial Alcohol, New York, N. Y., under the trade name, Vacatone. The guaranteed analyses is: protein, not less than 10 per cent; fat, not less than trace; crude fiber, not more than 1 per cent; nitrogen free extract, not less than 54 per cent; ash, not more than 33 per cent.

The cows were then returned to pasture for 28 days. The grass at this time (September) was less abundant than earlier and was supplemented with the previously fed riboflavin-low grain mixture. Finally, the animals were changed to the winter herd ration consisting of hay, phosphoric acid grass silage and the regular concentrate mixture described earlier. Riboflavin determinations were made for a period of 18 days while the cows were on this ration.

The results are summarized in table 1. The individual figures obtained with a given ration are presented as averages, in the interest of conserving space, since the significant periodic variations can be briefly described. Milk yield and total riboflavin output are recorded for consideration in discussing concentration figures.

TABLE 1

The influence of various rations on the concentration of riboflavin in milk. First experiment

Ration	Number of analyses	Length of period	Average daily production					
			Cow 5H			Cow 6H		
			Milk	Riboflavin		Milk	Riboflavin	
		days	*liters*	*mg. per liter*	*mg. per day*	*liters*	*mg. per liter*	*mg. per day*
Pasture	14	10	12.5	1.9	23.8	14.7	1.6	23.3
Riboflavin-low	64	62	10.6	1.5	15.9	14.2	1.2	17.0
Riboflavin-low plus molasses-yeast by-product	22	45	9.4	1.5	14.1	12.4	1.3	16.1
Riboflavin-low plus pasture	12	28	9.4	1.7	15.1	12.4	1.5	18.6
Normal herd	8	18	9.8	1.8	17.6	13.0	1.5	19.5

For each cow the riboflavin content of the milk varied little during the pasture period. It dropped to a lower level when the change was made to the vitamin-low ration, with the result that on the average the concentration was from 20 to 25 per cent less than during pasture feeding. With cow No. 6H this drop occurred with little change in milk yield, but in the case of the other cow there was a decrease in milk yield also and thus a drop in the total riboflavin output of over 30 per cent. The addition of the riboflavin supplement resulted almost immediately in an increase of about 20 per cent in the vitamin concentration in the milk, without change in milk yield, but, surprisingly, this increase was only temporary. At the end of two weeks it had disappeared. Thus, for the period as a whole, increasing the intake of riboflavin by 30 per cent and by an amount which was approximately equal to the amount previously being secreted, had an insignificant effect on the concentration of the vitamin in the milk. The total output was actually less, due to the drop in milk yield.

It is noted that when the cows were returned to pasture both the concentration and the total output of riboflavin markedly increased. The concentration did not reach the level found during the first pasture period. This is explainable on the basis that during the second period a considerable part of the ration consisted of the vitamin-low concentrate mixture. When the cows were changed to the winter herd ration there was a further increase in the riboflavin concentration and total output. Undoubtedly, this ration with its high quality hay and acid silage was rich in the vitamin.

In view of the rather surprising finding that the marked increase in riboflavin content when the molasses-yeast byproduct was added was only temporary, this observation was subjected to a further test. Six cows were used. Some initial data were obtained while they were on a fairly green October pasture, supplemented with good quality hay and the regular herd concentrate mixture. The cows were then changed to the winter ration described above. Finally, observations were made during a period when the molasses-yeast byproduct was included as 10 per cent of the concentrate mixture. The data obtained are presented in table 2.

TABLE 2

The influence of various rations on the concentration of riboflavin in milk. Second experiment

Cow number	Number of analyses	Duration of period	Average daily production		
			Milk	Riboflavin	
			liters	*mg. per liter*	*mg. per day*
(Pasture)					
3H	3	6	11.8	2.4	28.3
4H	3	6	14.2	2.1	29.8
5H	3	6	17.6	1.5	26.4
6H	3	6	21.1	1.7	35.9
7J	12	21	9.1	1.7	15.5
8H	12	21	12.4	1.5	18.6
(Herd ration)					
3H	17	51	9.8	2.3	22.5
4H	17	51	11.9	1.7	20.2
5H	17	51	16.7	1.5	25.0
6H	38	55	14.5	1.7	24.7
7J	20	50	9.8	1.8	17.6
8H	8	20	12.9	1.5	19.3
(Herd ration plus molasses-yeast)					
3H	20	62	7.7	2.6	20.0
4H	11	30	4.8	1.7	8.2
5H	20	62	13.3	1.5	20.0
6H*	....	....	....	....	....
7J	12	37	8.1	1.7	13.8
8H	6	20	10.8	1.8	19.4

* Cow 6H was dried off during the last period and thus no data are available.

It is noted that the change from pasture to the winter herd ration did not result in any significant decrease in riboflavin content of the milk,

except in the case of cow No. 4. The decreases in total riboflavin output noted with two other cows reflected primarily the drop in yield. On the other hand, the total riboflavin output increased slightly in the case of two of the cows, as a result of the changes in ration. When the molasses-yeast byproduct was added, increasing the riboflavin intake by about 10 per cent, an increase in the vitamin content in the milk as much as 20 per cent immediately resulted in all cases. Again, however, this increase proved temporary except in the case of two cows (No. 3H and No. 8H). In the case of the others, this temporary increase was sufficiently offset by a later decrease so that the average figures for the two periods came out about the same. It is noted that the increase in concentration recorded for cows No. 3H and No. 8H were not sufficient to keep up the total daily output in the milk.

A correlation of riboflavin with fat content of milk has been reported by Whitnah (10) and it was suggested that riboflavin may be concerned with the synthesis of milk fat. Hand and Sharp (4), however, have shown that an inverse relationship exists between the volume of milk secreted and the concentration of riboflavin. These workers point out that since both milk fat and riboflavin exhibit an inverse relation to milk yield the correlation between fat and riboflavin may not be a functional relationship.

TABLE 3

The relation of riboflavin secretion to the yield of milk and fat

Treatment of cow	Length of period	Average daily yield of milk, fat and riboflavin				
		Milk yield	Fat*	Fat	Riboflavin	
					Mg. per liter†	Daily total
	days	*liters*	*per cent*	*ml.*		
Herd ration	5	12.9	3.5	452	1.5	19.4
Herd ration plus 400 ml. cod liver oil daily	16	9.7	2.8	273	2.4	23.3
Herd ration	18	10.8	.....	.....	2.2	23.8
Herd ration plus 0.66 lb. butter fat daily	14	13.3	3.7	492	1.7	22.6
Herd ration	14	12.2	3.5	427	1.6	19.5

* Milk fat was determined on composite samples for the period.

† Riboflavin analyses were made each day on a sample from each milking. The data presented are averages for the period.

An unusual opportunity for testing this question was presented by another experiment which was in progress at the time that the previously described studies were being made. The experiment in question dealt with the influence of cod-liver oil in lowering milk fat percentage. By testing the milk samples for riboflavin the data presented in table 3 became available. It is seen that as the milk and fat yield declined the riboflavin increased, effecting an actual rise in the total daily excretion of riboflavin.

The feeding of cod-liver oil to the cow caused the fat percentage to drop one fifth and the daily fat secreted to decline almost 40 per cent. This drop in fat yield concurrent with an increase in the riboflavin excreted is good evidence that riboflavin is not related to the synthesis of milk fat. On the other hand, the data present a striking example of the inverse relation between milk yield and riboflavin content. Further evidence on this relation is presented by the data obtained in the goat studies described later.

DISCUSSION OF RESULTS

The two cow experiments show clearly that the riboflavin content of milk can be influenced by diet. In this respect they are in agreement with recently published data. Virtanen and Holmberg (9) found that cows fed fresh grass produced 30 per cent more riboflavin in the milk than cows fed hay and grain. Feeding A.I.V. silage along with the hay and grain maintained the riboflavin at the pasture level. Whitnah *et al.* (10) indicated that slightly higher values were obtained after cows had been on pasture. Kramer *et al.* (6) reported a 25 per cent increase in the riboflavin of milk on changing cows from dry summer conditions to green pasture later in the fall. They found no significant difference, however, between winter rations and pasture the following spring. Hand and Sharp (4) found that commercial summer milk contained about 20 per cent more riboflavin than winter milk.

Results here reported are of special interest, however, in indicating that even on a ration designed to be very low in riboflavin its content in the milk is not decreased more than 25 per cent compared to the level occurring on a ration rich in the vitamin. The marked yet temporary increases resulting from augmenting the riboflavin intake from a molasses-yeast by-product are also of interest, particularly the temporary effect which is unexplainable.

STUDIES WITH GOATS

In order to test the effect of lower intake levels of riboflavin than were possible in the cow experiments, purified diet studies with goats were undertaken. The following basal diet, designed to supply only minimum amounts of the vitamins of the B-complex, was used: vitamin-free casein, 15 parts; sucrose, 15 parts; corn starch, 40 parts; regenerated cellulose, 20 parts; cottonseed oil, 5 parts; mineral mixture, 5 parts; 0.25 per cent of a cod-liver oil concentrate to supply vitamins A and D. In the course of the experiments this basal diet was supplemented by the molasses-yeast by-product or by riboflavin alone. Six goats were studied during an entire lactation and one of them was used for study during a second lactation.

The milk yield and the riboflavin content of the milk is shown in figure 1. Riboflavin analyses were generally made every other day on milk samples taken at each milking. After determinations had been made during a

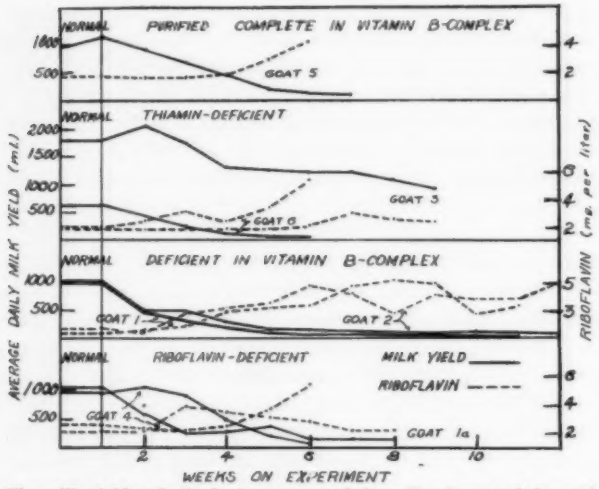

FIG. 1. The milk yield and riboflavin content of the milk of goats fed purified diets.

period of one week when the animals were still receiving a normal ration of hay and grain, they were transferred to the experimental diets. Goat 5 was fed the basal ration plus 10 parts of the molasses-yeast byproduct added, to supply factors of the vitamin B complex. Goats 3 and 6 were fed the basal ration supplemented with yeast, autoclaved to be free of thiamin. Goats 1 and 2 were fed the basal ration alone. Goats 1a and 4 were fed the basal ration supplemented with tikitiki to supply factors of the vitamin B complex except riboflavin.

The milk studied contained from 1.3 to 6.5 mg. of riboflavin per liter. In most cases a rapid decline in milk yield was associated with a marked increase in the riboflavin concentration. The total riboflavin secreted per day was less, however, as the milk flow declined. As the goats became almost dry the content of riboflavin in the milk often fell to the original low level.

The inverse relationship between the milk yield and the concentration of riboflavin is strikingly shown in figure 1. This same inverse relationship was noted earlier in the studies with cows.

The milk yield of all the goats studied dropped rapidly when they were transferred from a normal diet of hay and grain to the purified diets. Food consumption was poor and it is thought that this was responsible for production failure rather than lack of the B vitamins. The fact that goat 5, fed the purified diet adequate in factors of the vitamin B-complex, failed in lactation as early as those receiving diets lacking in thiamin, riboflavin or the entire B-complex supports this view. Evidence that goats have no

dietary requirement for riboflavin as an essential for either maintenance or lactation is presented in the fact that the animals fed diets free from riboflavin produced milk containing fully as much of this vitamin as other goats receiving an abundant supply in the feed. After six months on the riboflavin deficient feed one goat continued to secrete appreciable quantities of this vitamin.

By application of chemical methods for the determination of thiamin and riboflavin, data (unpublished) have been obtained in this Laboratory indicating that both riboflavin and thiamin are formed in the rumen. Analyses show that when goats, sheep and calves were fed diets devoid of thiamin and riboflavin the rumen contents contained one to three micrograms of thiamin and 6 to 20 micrograms of riboflavin per gram of dry material. These data confirm and extend the work of McElroy and Goss (7) who showed that the rumen contents of sheep were richer than the diet fed with respect to four factors of the vitamin B-complex. Theiler (8) and Bechdel *et al.* (1, 2) maintained ruminants for extended periods upon diets low in vitamin B. Under these conditions the latter workers demonstrated the presence of vitamin B in the rumen contents of cattle. Until recently, however, no evidence has been presented to indicate how many factors of the vitamin B-complex are formed in the rumen. To our knowledge this is the first evidence of the synthesis of riboflavin and thiamin in the rumen of animals when they are fed purified diets devoid, or practically so, of these factors.

SUMMARY

Experiments with cows, involving 460 determinations made over a period of 9½ months, showed clearly that the riboflavin content of milk can be influenced only to a limited extent by the diet. When cows were transferred from pasture to a ration of natural feeds selected to be very low in riboflavin its content in the milk decreased about 25 per cent. Increasing the riboflavin intake 30 to 50 per cent by feeding a molasses-yeast byproduct caused only a temporary increase in the concentration of riboflavin in the milk. A winter ration consisting of good quality hay, acid grass silage and a grain mixture maintained the milk riboflavin at the pasture level.

Goats fed a riboflavin-free purified diet continued to secrete large amounts of riboflavin in the milk, indicating this factor is not a dietary essential for lactation in the goat. No consistent difference was observed in the milk yield or the riboflavin concentration when the purified diet was supplemented with a molasses-yeast byproduct supplying riboflavin. Likewise, no advantage in milk secreted was noted between a thiamin-deficient purified diet and one adequate in it and other factors of the B-complex.

The data from both cows and goats indicate that there is an inverse relation between the milk yield and the riboflavin concentration of the milk.

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OFFICIAL BODY AND TEXTURE CRITICISMS OF DAIRY PRODUCTS JUDGED IN THE NATIONAL CONTEST

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As similarly pointed out in a previous report (1) this analysis of the official judgments of the samples of dairy products scored in the Students' National Contest in the Judging of Dairy Products is made: 1, that coaches may appreciate more fully the body and texture defects recurring most frequently in the various dairy products, 2, that they may realize the association and combination of body and texture defects encountered, 3, that they may have an understanding of the relationship between the body and texture quality and the number of criticisms made, and 4, that the student may gain encouragement from the fact that although many specific body and texture defects are possible in dairy products those most likely to be encountered in the judging of normal salable dairy products are relatively few in number. The period in the study extends from 1926 to 1939, inclusive, covering 14 national contests in which 104 samples of butter, cheese, ice cream, and milk were scored. Obviously, consideration is not given to milk in this report as body and texture has no part in the official score card of milk.

In the previous report (1) it was pointed out that prior to 1930, two or more official judges were selected to place scores and criticisms on each product. Their services were not continued from year to year, although one judge may have served more than one year. Beginning in 1930, with few exceptions, the official criticisms have been placed on the samples by a selected judge for each product, and, beginning in 1932, two "coach" judges have assisted. If the three failed to agree in their judgment of the sample, that sample was not used in the contest. As noted in "History and Development of the Students' National Contest in the Judging of Dairy Products" (2) the official judges have been retained in the same capacity each year, but the same "coach" judges have not been used continuously from year to year. Consequently, the body and texture criticisms placed on the samples should represent as reliable a composite judgment as it is possible to obtain. However, it must be borne in mind that probably in some cases specific samples exhibiting certain body and texture defects were selected for use in the contest and therefore the percentage distributions reported herein may not apply to commercial products as a whole.

BUTTER

A study of the official body defects of butter from 1926 to 1939, in-

Received for publication August 12, 1940.

clusive, shows that an average of 1.13 criticisms was made per sample criticized. However, only 15, or 14.4 per cent, of the 104 tubs of butter scored were criticized on body.

The distribution of the body defects noted in these few samples of butter are presented graphically in figure 1. The data show that 29.4 per cent of the body criticisms were "leaky"; 23.5 per cent "crumbly"; 17.6 per cent each were "weak" and "sticky"; and 5.9 per cent each were "mealy" and "salvy." Leaky and weak-bodied butter was noted throughout the period of this judging, whereas, crumbly and sticky butter has been encountered particularly within recent years.

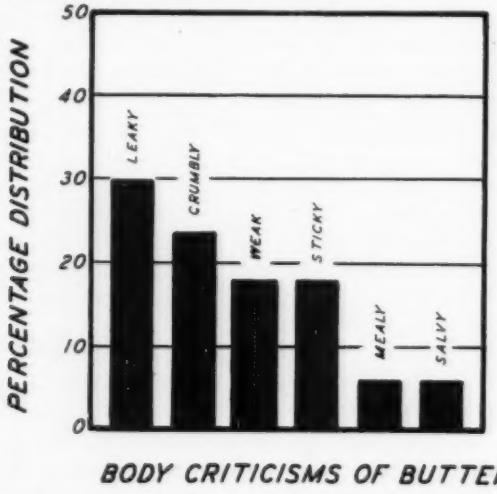

FIG. 1. Distribution of official body criticisms of samples of butter judged in the Students' National Contest in the Judging of Dairy Products 1926 to 1939, inclusive.

Apparently the defects were obvious before the score for body was cut and then the cut made was only one-half of one point. Two samples were criticized slightly on body, but merited no cut; the remaining samples criticized each scored 24.5 points on body.

When the body of the butter was found to be defective a single, specific criticism was usually made, such as "leaky," "crumbly," or "sticky." However, two samples each merited two criticisms which were "sticky, weak" and "salvy, weak."

Emphasis should be placed on the fact here that over 85 per cent of the samples of the butter were scored perfect on body. However, it must be brought out that a few samples were criticized and cut on body with the criticisms as given above and as shown graphically in figure 1.

The butter was scored by L. E. Gaylord and H. W. Gregory in 1926;

by L. S. Edwards and G. A. Gilbert in 1927 and 1928; by L. S. Edwards and H. D. Reynolds in 1929; by H. D. Reynolds and O. A. Storvick in 1930; by H. D. Reynolds and L. D. Reekie in 1931; by C. E. Eckles assisted by coaches E. S. Guthrie and R. E. Roberts in 1932; by C. L. Pier assisted by coaches L. C. Thomsen and F. H. Herzer in 1933; by L. S. Edwards from 1934 to 1938 inclusive, assisted each year by two of the following coaches: M. Mortensen, R. E. Roberts, S. L. Tuckey, E. S. Guthrie, L. C. Thomsen, C. M. Mecham, N. E. Fabricius, E. O. Herreid, and S. T. Coulter; and by B. J. Ommodt assisted by N. E. Fabricius and C. C. Totman in 1939. Thus the official judging was done during the period of this study by ten official judges and eleven coach judges.

CHEESE

During the fourteen-year period from 1926 to 1939, inclusive, 94 samples, or 90.4 per cent, of the 104 samples of cheese used in the scoring contests were criticized on body and texture. A study of the data shows that an average of 1.67 body and texture criticisms was made for each sample so criticized. The distribution of these body and texture criticisms is presented in figure 2.

Of the many possible body and texture criticisms, that of "open" was used in 44, or 28 per cent, of the 157 criticisms. "Weak," "mealy," and "pasty" designated the body and texture defects of 15.9, 12.7 and 12.7 per cent of the criticisms respectively. "Curdy" or "corky" and "gassy" each accounted for 6.4 per cent of the criticisms. These six criticisms accounted for 82.1 per cent of all the criticisms. The remaining 17.9 per cent was divided among five other body and texture defects, "crumbly," "sweet curd holes," "yeast holes," "flaky," and "lumpy." A "lumpy" or "uneven" textured cheese was encountered in only one of the 104 cheese used in official judging.

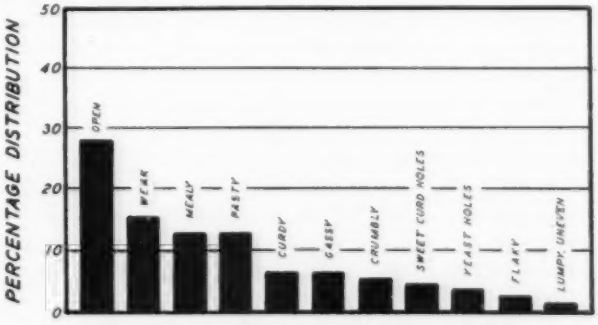

FIG. 2. Distribution of official body and texture criticisms of samples of cheese judged in the Students' National Contest in the Judging of Dairy Products, 1926 to 1939, inclusive.

Combinations of body and texture defects encountered were "open, weak," "open, pasty," "open, mealy," "open, crumbly," "gassy, weak," "gassy, curdy," "pasty, weak" and so on. Occasionally the body and texture was so poor as to merit three or more criticisms. In such cases combinations as "mealy, open (gassy)," or "gassy, open, pasty," or "pasty, open (weak, gassy)" were used.

The average body and texture score of the 104 samples was 27.7. This takes into consideration the low body and texture scores of three samples used in the 1926 contest, which were 23, 25, and 25.5 points, slightly under the present limits now recognized in scoring the body and texture of cheese. Not since 1928 when two samples were scored perfect, 30 points, have any samples been scored perfect in body and texture. During the past five contests, 1935 to 1939, inclusive, the average body and texture scores have been 28.0, slightly higher than the average of 27.3 for the five years prior to that period.

The cheese was judged in 1926 by C. W. Freyhoffer, and E. L. Aderhold; in 1927 by G. N. Tobey and G. A. Gilbert; in 1928 by G. N. Tobey, G. A. Gilbert, and L. H. Marlatt; in 1929 by H. L. Wilson and William White; in 1930 by H. L. Wilson and J. W. Moore; and in 1931 by H. L. Wilson and W. E. Ayres. From 1931 to 1938 inclusive the cheese was judged by H. L. Wilson assisted each year by two of the following coaches: W. H. Martin, E. F. Goss, P. A. Downs, G. M. Trout, F. W. Bennett, C. A. Jacobson, R. E. Roberts, S. T. Coulter, H. G. Lindquist, W. H. Sproule, S. L. Tuckey, L. C. Thomsen, and K. M. Renner; and in 1939 by F. H. McCampbell assisted by L. C. Thomsen and C. A. Phillips. A total of ten official judges and fourteen coach judges have placed judgments on the cheese scored from 1926 to 1939 inclusive.

ICE CREAM

During the fourteen-year period from 1926 to 1939, inclusive, 77 samples, or 74 per cent, of the 104 official ice cream samples in the contests were criticized on body and texture. For each sample criticized on body and texture an average of 1.90 criticisms was given. The percentage distribution of these criticisms is presented in figure 3.

The chief body and texture defect of the frozen product was "coarse" being noted in 22.6 per cent of the criticisms, whereas "curdy, wheys off," comprising 18.0 per cent of the criticisms, was the chief criticism of the melted product. Closely following the coarse texture in percentage distribution was the "weak" body with 15.8 per cent. The criticisms "crumbly," "does not melt," "icy," "soggy," and "fluffy" were fairly well divided, each present 9.6, 8.9, 8.2, 8.2, and 6.2 per cent respectively. Three samples, or 2.1 per cent of those criticized were criticized for being "buttery."

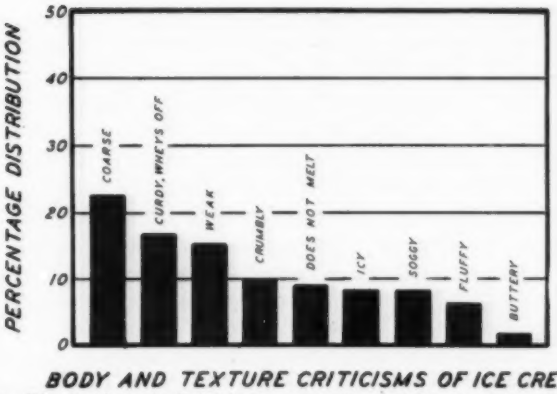

FIG. 3. Distribution of official body and texture criticisms of samples of ice cream judged in the Students' National Contest in the Judging of Dairy Products, 1926 to 1939, inclusive.

Since an average of almost two criticisms per sample was used to criticize the body and texture of the samples, obviously with some samples three or more criticisms were used to describe the defect. The body and texture of one sample was described as "coarse, icy, weak, curdy, does not melt"; another was "buttery, coarse, crumbly, weak, grainy, curdy." However, for the most part such combinations as "coarse, curdy," "coarse, weak," "coarse, crumbly," "icy, weak," or "curdy, does not melt," seemed to prevail.

The average body and texture score of 99 samples (5 were not included because they were too soft to score in the frozen condition) was 22.97; that only of the 77 samples criticized was 22.6; whereas, that of the 22 without criticism was 24.4. Since 1932, when five samples were allowed perfect score on body and texture, no samples have been given a perfect score of 25 on body and texture. Prior to 1931 samples were scored for body and texture as low as 17 or 18. However, since then, the lower limit of body and texture score for ice cream has been established at 20. The mean body and texture score per year for all samples has varied from 23.0 in 1935 to 23.7 in 1939. This does not necessarily indicate a trend toward higher scores for body and texture as the mean score in 1938 was 23.1.

During the period of the study the ice cream samples were officially judged one year by H. F. Judkins and C. S. Trimble; one year by H. F. Judkins; two years by W. H. E. Reid; one year by P. H. Tracy; two years by A. C. Dahlberg and A. D. Burke; seven years by A. C. Dahlberg assisted by two of the following coaches: P. H. Tracy, R. W. Smith, C. A. Iverson, E. L. Fouts, W. H. Martin, J. H. Erb, F. H. Herzer, P. S. Lucas, L. R. Dowd, P. A. Downs, G. M. Trout and N. E. Fabricius; and last year by

F. W. Milner, assisted by coach judges S. T. Coulter and M. G. Pederson. Thus the official judging was done during the period of this analysis by seven official judges and fourteen coach judges.

SUMMARY

A study of the official body and texture criticisms of butter, cheese, and ice cream samples used in the Students' National Contest in the Judging of Dairy Products during the fourteen-year period from 1926 to 1939, inclusive, showed: 1, that certain body and texture defects are more current than others; 2, that while butter is comparatively free of body and texture defects some occur infrequently; 3, that body and texture defects are very frequently encountered in the greater majority of samples of cheese and ice cream; and 4, that some association of defects exists in those products.

Predominating body and texture criticism of butter was "leaky"; those of cheese were "open," "weak," "pasty" and "mealy," and of ice cream "coarse" and "weak" for the frozen ice cream and "curdy" for the melting appearance. One criticism seemed adequate to describe any body and texture defect of butter, but an approximate average of two was necessary to describe properly the body and texture defects of cheese and ice cream.

As in the previous report (1) the result of this study and analysis of trends in official body and texture judgments is in no way intended as a guide to future scoring, but merely to classify and make available the body and texture criticisms for those who may not have access to the official scoring records as well as calling attention to this phase of dairy products judging. Inasmuch as the samples used may have been selected in many cases for a specific defect, the percentages distribution reported herein may not necessarily apply to commercial products as a whole. However, the body and texture criticisms encountered in these studies appear to be representative of those encountered in the commercial products throughout the country.

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SOME FACTORS AFFECTING THE STABILITY OF CERTAIN MILK PROPERTIES. IV. A COMPARISON OF SEVEN DIFFERENT ROUGHAGES ON THE COLOR AND FLAVOR OF MILK*

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Considerable attention during the past 4 or 5 years has been focused on the importance of roughages in producing winter milk of good flavor and color. Most of the attention of research workers seems to stem from the important observations reported by Anderson (1, 2) and his co-workers. The idea that high-carotene feeds are associated with good milk flavor is not, however, as modern as one might suppose, because in 1856 Rebecca A. Upton (3) wrote in a book which she called "Home Studies" that, "Carrots give the finest color and flavor to milk, and consequently to butter." She refers to the carrot as the "especial ally of the dairy."

Since Anderson's first work, several other investigators (4, 5, 6, 7, 8, 9, 10, 11, 12) have directed attention to the importance of high-carotene roughages in producing milk of good flavor. Following up work (4, 13) reported earlier, three separate experiments have been conducted on the effect of dairy roughages on the color and flavor of milk and are discussed in this paper.

COMPARISON OF THE EFFECTS OF MOLASSES ALFALFA SILAGE AND OF SPRING PASTURE ON MILK COLOR AND FLAVOR

Six Holstein and 14 Guernsey cows were fed for a period of 17 weeks on a good grade of alfalfa silage preserved with commercial molasses at the rate of 100 lbs. of molasses per ton of green material. This feeding period was followed by a period of 3 weeks on spring pasture. The cows had been on corn silage for several weeks when the experiment began. The hay and grain rations were kept as constant throughout the experiment as was practicable. Samples of milk representative of one day's complete milking were taken biweekly from each cow and were pasteurized in Pyrex glass flasks under conditions which duplicated as nearly as possible the holding method used in commercial practice. Each sample, which amounted to about one quart, was subdivided and each subdivision was analyzed for color and flavor while fresh, at the end of 48 hours and at the end of 96 hours. Copper sulfate, at the rate of 0.5 and 2 parts of copper per million parts of milk, was added to half of those subdivisions which were to be analyzed after 48 and 96 hours.

Received for publication August 22, 1940.

* Journal series paper of the New Jersey Agricultural Experiment Station, Department of Dairy Husbandry.

The results on color are presented in table 1 and figure 1.

TABLE 1

Effect of molasses alfalfa silage and spring pasture on the yellow color of milk*

	Average initial color		Average color after 96 hours		
	Raw	Pasteurized	No Cu	0.5 p.p.m. Cu	2 p.p.m. Cu
Holsteins					
Begin. of expt.	3.50	3.44	3.38	3.34	3.29
Ave. of silage	4.72	4.69	4.59	4.50	4.47
End of silage	5.13	5.12	4.98	4.95	4.95
Ave. of pasture	5.65	5.59	5.47	5.38	5.36
Guernseys					
Begin. of expt.	5.11	5.04	4.99	4.91	4.88
Ave. of silage	6.33	6.27	6.20	6.16	6.11
End of silage	6.65	6.59	6.56	6.51	6.45
Ave. of pasture	6.72	6.68	6.61	6.55	6.51

* Expressed as lactochrometer units.

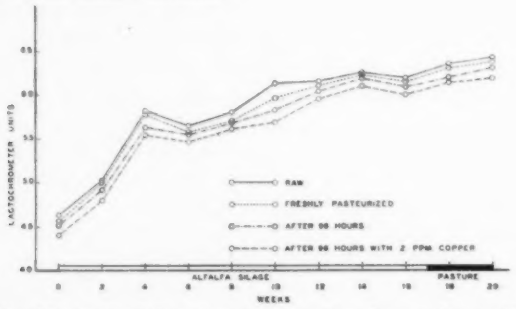

FIG. 1. Biweekly trend of the color of milk produced on molasses alfalfa silage and on early spring pasture.

These results indicate that a change from a diet of alfalfa silage to one of pasture caused only a slight increase in color. This increase was too small to be detected by visual observation. Actually, most of this slight increase probably is due, not directly to a change in feed, but more likely to a decrease in milk production and an increase in fat content. Previously reported results (11) showed that a highly significant positive correlation exists between yellow color and fat content in milk. By referring to figure 2 it is seen that an abrupt rise in fat content occurred when the cows were put on pasture.

The week-by-week trend of the color of the milk while the cows were on alfalfa silage shows that most of the increase occurs in the first 4 weeks of feeding but that the maximum is not reached until about the tenth week. Further increases in color after the tenth week probably are due to increases in fat content.

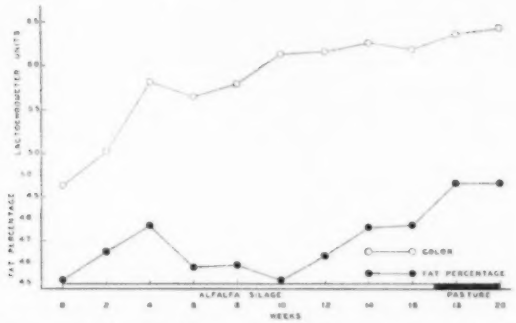

FIG. 2. Relation of the color to the fat percentage of milk.

The data presented in table 1 and in figure 1 show that there is a progressive loss of color while the milk is in storage and that this loss is accelerated by pasteurization and further by the action of soluble copper. These results substantiate previously reported ones (13).

TABLE 2

Effect of molasses alfalfa silage and of spring pasture on the flavor of milk

	Average flavor score		
	Initial	After 48 hours	After 96 hours
Beginning of experiment	20.54	20.03	19.70
Ave. of silage period	22.15	21.69	21.02
End of silage period	22.50	21.96	21.21
Ave. of pasture period	21.89	21.37	20.47

The effect of alfalfa silage and of pasture on the flavor of milk is shown by the data in table 2 and in figure 3. When the cows were changed from corn silage to alfalfa silage at the beginning of the experiment there was a decided rise in flavor score. A high level of good flavor was maintained until the cows were put on pasture, at which time there was a slight drop. This drop can be attributed mainly to the occurrence of "grassiness" in the milk. Throughout the alfalfa silage feeding period the milk from each cow almost invariably had a mellow and very fine flavor. The characteristic flavor of milk produced on grass or legume silage is quite distinct from that produced on corn silage or beet pulp and resembles very closely the flavor of milk produced on weed-free pasture grasses but does not have the characteristic objectionable "grassy" flavor.

Copper in concentrations of 0.5 and 2 parts per million parts of milk was added to subdivisions of each sample of milk in order to determine the resistance of the milk toward the development of metal-induced oxidized flavor. The average results presented in table 3 and in figure 4 show that

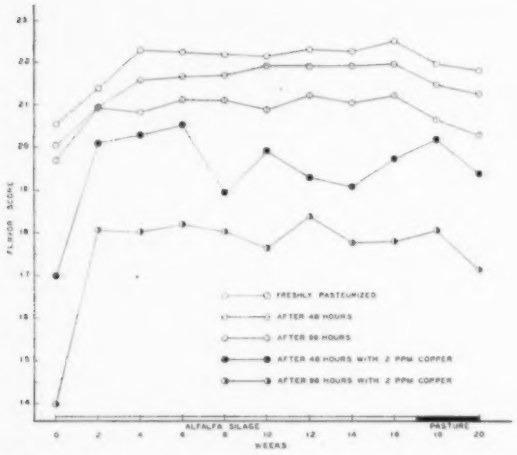

FIG. 3. Biweekly trend of the flavor score of milk produced on molasses alfalfa silage and on early spring pasture.

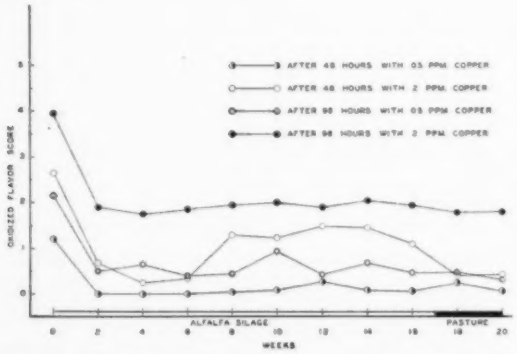
AFTER 48 HOURS WITH .05 PPM COPPER
AFTER 48 HOURS WITH .2 PPM COPPER
AFTER 96 HOURS WITH .05 PPM COPPER
AFTER 96 HOURS WITH .2 PPM COPPER
OXIDIZED FLAVOR SCORE
ALFALFA SILAGE
PASTURE
WEEKS

FIG. 4. Biweekly trend of the intensity of copper-induced oxidized flavor in milk produced on molasses alfalfa silage and on early spring pasture.

milk produced on alfalfa silage has high resistance and this resistance is equal to that of the milk produced on spring pasture. There was not a single instance of oxidized flavor in milk not purposely contaminated with copper. At the end of 48 hours there were many instances where copper-contaminated milk had no oxidized flavor even though the milk was pasteurized and contained as much as 2 parts of copper per million parts of milk.

EFFECT OF MOLASSES GRASS SILAGE, BEET PULP AND CITRUS PULP ON COLOR AND FLAVOR

Two groups of 6 cows each, matched with regard to breed and as nearly

TABLE 3

Effect of molasses alfalfa silage and of spring pasture on the development of copper-induced oxidized flavor in milk

	Average intensity* of oxidized flavor			
	After 48 hours		After 96 hours	
	0.5 p.p.m. Cu	2 p.p.m. Cu	0.5 p.p.m. Cu	2 p.p.m. Cu
Beginning of experiment	1.20	2.65	2.15	3.95
Ave. of silage period	0.07	0.98	0.58	1.93
End of silage period	0.06	1.10	0.47	1.94
Ave. of pasture period	0.13	0.40	0.37	1.79

* 0 = not present. 0.5 = doubtful. 1 to 5 = degrees of intensity.

as possible with regard to milk production, were fed according to the following plan:

Period of feeding	*Group I*	*Group II*
4 weeks	Beet pulp	Grass silage
1 week	Transition	Transition
4 weeks	Citrus pulp	Citrus pulp
1 week	Transition	Transition
4 weeks	Grass silage	Beet pulp

The dried citrus pulp was impregnated with commercial molasses to make it more palatable. The grass silage was not of first quality since it was produced during dry weather and contained a considerable proportion of weeds. The hay and grain rations were kept as nearly constant as was possible throughout the experiment.

Samples representative of one day's milking were taken weekly from each cow and were pasteurized as previously described. The samples were subdivided and were analyzed for color and flavor while fresh and at the end of 72 hours. Copper sulfate at the rate of 1.3 parts of copper per million parts of milk was added to one of the subdivisions of each sample to be used for judging at the end of 72 hours.

TABLE 4

Effect of molasses grass silage, of beet pulp and of citrus pulp on the color of milk*

Feeding period	Average lactochrometer units		
	Group I	Group II	Groups I and II
Ave. of beet pulp period	4.88	4.67	4.78
End of beet pulp period	4.68	4.55	4.62
Ave. of citrus pulp period	4.52	4.99	4.76
End of citrus pulp period	4.37	4.83	4.60
Ave. of grass silage period	5.43	5.56	5.50
End of grass silage period	5.79	5.86	5.83

* Expressed as lactochrometer units.

Since the periods were short and there was some "carry-over" effect of one feed into the next feeding period the color values are given in table 4 as the average for each period and for the end of each period. Beet pulp and citrus pulp seem to be about equal in their effect on milk color; both of them greatly lower the color as compared to grass silage if fed for a sufficient length of time. The week-by-week trend of the milk color for both groups is shown in figure 5.

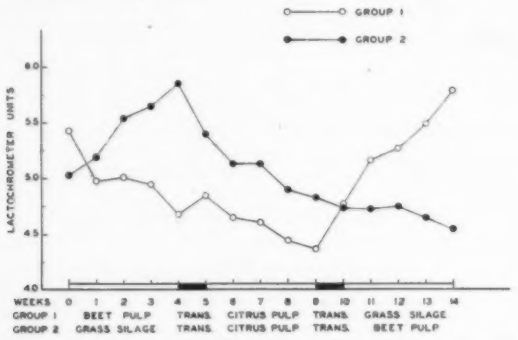
GROUP 1
GROUP 2
LACTOCHROMETER UNITS
6.0
5.5
5.0
4.5
4.0
WEEKS 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
GROUP 1 BEET PULP TRANS. CITRUS PULP TRANS. GRASS SILAGE
GROUP 2 GRASS SILAGE TRANS. CITRUS PULP TRANS. BEET PULP

FIG. 5. Weekly trend of the color of milk produced on 3 types of succulent roughage.

TABLE 5

Effect of molasses grass silage, of beet pulp and of citrus pulp on the flavor of milk

Feeding period	Average flavor score					
	Group I		Group II		Groups I and II	
	Fresh	72 hrs.	Fresh	72 hrs.	Fresh	72 hrs.
Ave. of beet pulp period	20.50	19.41	20.52	19.69	20.51	19.55
End of beet pulp period	20.38	19.17	20.50	19.67	20.44	19.42
Ave. of citrus pulp period	20.59	19.27	20.57	19.75	20.58	19.53
End of citrus pulp period	20.33	19.08	20.54	19.42	20.44	19.25
Ave. of grass silage period	21.72	20.98	21.73	20.91	21.73	20.95
End of grass silage period	22.08	21.46	22.08	21.46	22.08	21.46

The flavor of the milk produced on either citrus pulp or beet pulp was inferior to that produced on grass silage as shown by the data presented in table 5 and in figures 6 and 7. A bitter flavor frequently appeared in the milk produced on citrus pulp which cleared up when the cows were changed to grass silage or beet pulp. Even though molasses was added to the citrus pulp, any great improvement in palatability was questionable since it was difficult to get most of the cows to eat quantities of it comparable to their consumption of beet pulp. Consequently, there was a drop in production and in turn there may have been other physiological disturbances which conceivably could have caused bitterness in the milk. It is

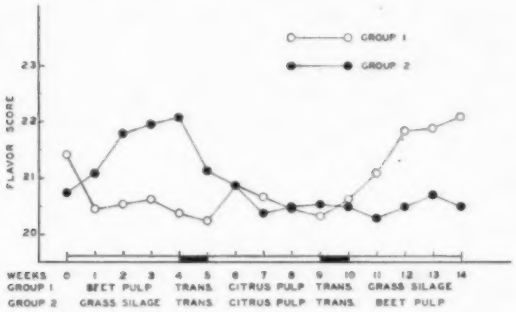
GROUP 1
GROUP 2
FLAVOR SCORE
23
22
21
20
WEEKS 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
GROUP 1 BEET PULP TRANS. CITRUS PULP TRANS. GRASS SILAGE
GROUP 2 GRASS SILAGE TRANS. CITRUS PULP TRANS. BEET PULP

FIG. 6. Weekly trend of the flavor score of freshly pasteurized milk produced on 3 types of succulent roughage.

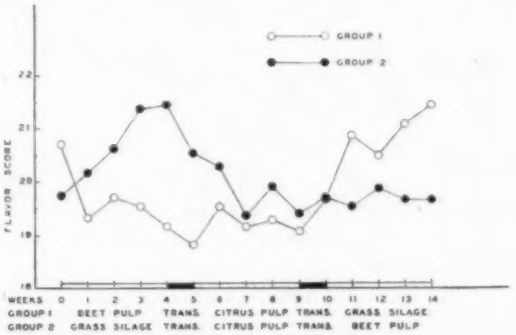
GROUP 1
GROUP 2
FLAVOR SCORE
22
21
20
19
18
WEEKS 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
GROUP 1 BEET PULP TRANS. CITRUS PULP TRANS. GRASS SILAGE
GROUP 2 GRASS SILAGE TRANS. CITRUS PULP TRANS. BEET PULP

FIG. 7. Weekly trend of the flavor score of 72-hour storage pasteurized milk produced on 3 types of succulent roughage.

possible that, if sufficient time were allowed, the cows eventually might come to like citrus pulp.

TABLE 6

Effect of molasses grass silage, of beet pulp and of citrus pulp on the development of copper-induced oxidized flavor in milk*

Feeding period	Average intensity of oxidized flavor after 72 hrs.		
	Group I	Group II	Groups I and II
Ave. of beet pulp period	4.04	4.21	4.13
End of beet pulp period	4.17	4.17	4.17
Ave. of citrus pulp period	4.00	3.46	3.73
End of citrus pulp period	4.33	3.83	4.08
Ave. of grass silage period	1.96	2.25	2.11
End of grass silage period	1.00	1.83	1.42

* Added 1.3 parts of copper per million parts of milk.

Based on the average oxidized flavor score of groups I and II combined it might be concluded that citrus pulp is a little superior to beet pulp in

producing milk resistant to metal-induced oxidation. It must be pointed out, however, that one of the citrus pulp feeding periods was immediately preceded by a period of feeding grass silage and there appears to be some "carry-over" effect. This is shown by the week-to-week trend of the intensity of the oxidized flavor presented in figure 8. It seems reasonable,

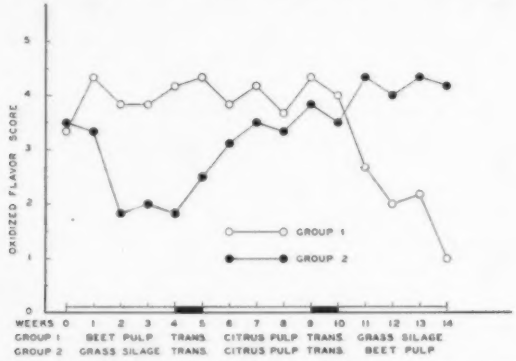
OXIDIZED FLAVOR SCORE
0
1
2
3
4
5
GROUP 1
GROUP 2
WEEKS 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
GROUP 1 BEET PULP TRANS. CITRUS PULP TRANS. GRASS SILAGE
GROUP 2 GRASS SILAGE TRANS. CITRUS PULP TRANS. BEET PULP

FIG. 8. Weekly trend of the intensity of copper-induced oxidized flavor in milk produced on 3 types of succulent roughage.

therefore, to accept the values at the end of each period for comparison and on this basis citrus pulp offers no significant advantage over beet pulp in producing resistant milk.

EFFECT OF MOLASSES GRASS SILAGE, PHOSPHORIC ACID GRASS SILAGE AND CORN SILAGE ON THE COLOR AND FLAVOR OF MILK

Workers (14) at the Massachusetts Agricultural Experiment Station reported that treatment with phosphoric acid resulted in a better preservation of the carotene in the ensiled material than did preservation with molasses. Unpublished data (15) of the New Jersey Agricultural Experiment Station also indicated that phosphoric acid was a better preserver of silage carotene than was molasses.[1] On the other hand, Camburn, Ellenberger and Crooks (16) reported that molasses and phosphoric acid preserved the carotene in ensiled grasses equally well.

A comparison of the effects on milk color and flavor was made between molasses grass silage, phosphoric acid grass silage, and corn silage by feeding continuously the 3 types of silage to 3 groups of 6 cows each for 18 weeks. The molasses silage group consisted of 3 Holsteins and 3 Guernseys, the phosphoric acid silage group, 2 Holsteins and 4 Guernseys, and the corn silage group, 3 Holsteins and 3 Guernseys. In order that the results between groups be comparable, all averages for the groups were calculated

[1] Permission to use these results was kindly given by M. W. Taylor and W. C. Russell of the Department of Agricultural Biochemistry.

by taking the mean of the averages for each breed. To make the two types of grass silage as nearly alike as possible with the exception of the preservative, loads of the green material from the same field were alternated between the 2 silos. Hay and grain rations were the same for all 3 groups. The cows had been on corn silage for several weeks previous to the start of the experiment.

Samples of milk representative of one day's milking were taken biweekly from each cow and were pasteurized by the usual laboratory method. Subdivisions of each sample were made in the usual way and copper sulfate at the rate of 1.3 parts of copper per million parts of milk was added to a portion of the subdivisions. Color of the pasteurized samples was analyzed and flavor of the samples, while fresh and after 72 hours, was scored.

TABLE 7

Effect of molasses grass silage, of phosphoric acid grass silage and of corn silage on the color of milk

	Lactochrometer units		
	M.G.S.	P.A.G.S.	C.S.
Beginning of the experiment	5.03	5.24	5.12
Lowest* weekly ave.	5.95	5.95	5.06
Highest* weekly ave.	6.42	6.38	5.24
Ave. for entire period	6.24	6.12	5.11
End of experiment	6.76	6.41	5.00

* Exclusive of the first 2 weeks and the last week.

Data presented in table 7 and in figure 9 show the effect of the 3 silages

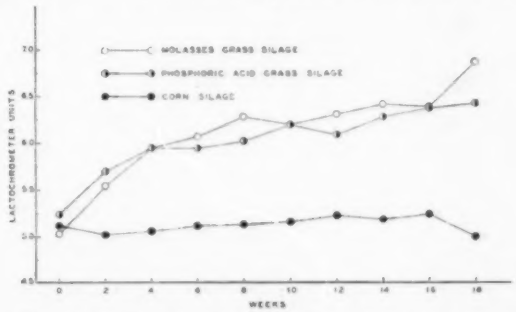

FIG. 9. Biweekly trend of the color of milk produced on 3 types of silage.

on milk color. The color values for the 3 groups at the beginning of the experiment are comparable although a slight advantage was held by the phosphoric acid and corn silage groups. Corn silage maintained the color of milk throughout the experiment at a point near the value for the group at the beginning of the experiment which was expected since the cows had

been on this same type of feed for a considerable period of time before the experiment began. Both types of grass silage greatly increased the color over the values obtained at the beginning of the experiment and, although the molasses group had a slightly higher average for the entire period than did the phosphoric acid group, it is doubtful that this difference is highly significant. Even though the phosphoric acid may show a better preservation of carotene in the silage than molasses the difference does not seem to be great enough to be reflected in the color of the milk produced on the 2 silages.

TABLE 8

Effect of molasses grass silage, of phosphoric acid grass silage and of corn silage on the flavor of milk

	Flavor score					
	M.G.S.		P.A.G.S.		C.S.	
	Fresh	72 hrs.	Fresh	72 hrs.	Fresh	72 hrs.
Beginning of experiment	20.71	19.00	20.82	18.91	20.88	19.84
Lowest* weekly ave.	21.79	20.73	21.63	20.78	20.21	19.00
Highest* weekly ave.	22.34	21.38	22.13	21.22	20.75	19.59
Ave. for entire period	22.09	20.97	21.91	20.85	20.57	19.39
End of experiment	21.78	20.09	21.96	20.34	20.00	19.13

* Exclusive of the first 2 weeks and the last week.

The effects of the 3 types of silage on the flavor of milk are shown in table 8 and in figures 10 and 11. Difference in the effects of the 2 types

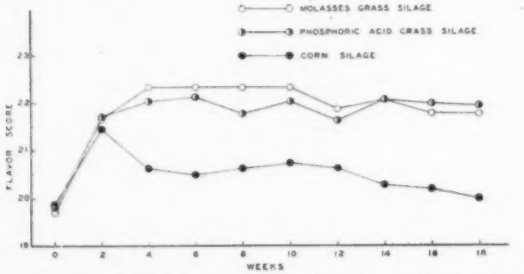

FIG. 10. Biweekly trend of the flavor score of freshly pasteurized milk produced on 3 types of silage.

of grass silage on the flavor is not marked and probably is not significant. Both were superior to corn silage. The slight drop in the flavor score of the milks from all groups at the end of the experiment most likely is due to the fact that many of the cows had neared the end of their lactation periods. The maximum flavor score attained by the two groups on grass silage in this experiment did not reach that of the milk of the cows on alfalfa silage. The alfalfa silage experiment was conducted during the

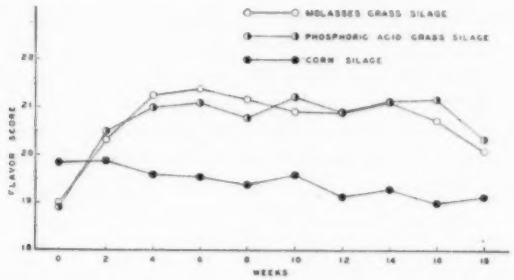

FIG. 11. Biweekly trend of the flavor score of 72-hour storage pasteurized milk produced on 3 types of silage.

winter of 1938–39 while this latter experiment was conducted in 1939–40. The growing season of 1938 was excellent for grass silage while the 1939 season was extremely dry and thus the quality of the green material which went into the silos was not first class. This may account partly for the lower average flavor scores of the milk produced in 1939–40.

TABLE 9

Effect of molasses grass silage, of phosphoric acid grass silage and of corn silage on the development of copper-induced oxidized flavor in milk*

	Intensity† of oxidized flavor		
	M.G.S.	P.A.G.S.	C.S.
Beginning of experiment	3.67	3.75	3.17
Lowest‡ weekly ave.	0.84	1.13	3.17
Highest‡ weekly ave.	1.67	1.75	3.67
Ave. for entire period	1.48	1.48	3.32
End of experiment	0.50	1.50	3.50

* Added 1.3 parts of copper per million parts of milk.
† 0 = not present. 0.5 = doubtful. 1 to 5 = degree of intensity. Judged after 72 hours.
‡ Exclusive of the first 2 weeks and the last week.

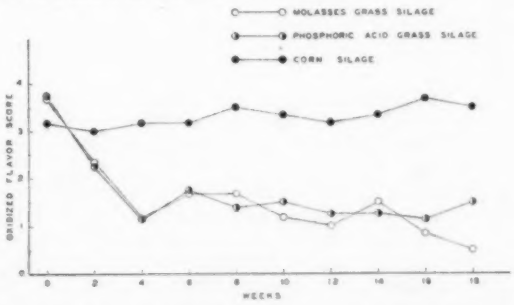

FIG. 12. Biweekly trend of the intensity of copper-induced oxidized flavor of milk produced on 3 types of silage.

The intensity of copper-induced oxidized flavor tended to increase with continued feeding of corn silage while that of the milk produced on both types of grass silage was greatly reduced as shown by the data presented in table 9 and in figure 12. It is interesting to note that the average intensity of the oxidized flavor of the milk produced on corn silage in this experiment and in a previous experiment (4) is approximately the same; 3.32 for this experiment and 3.42 for the previous experiment.

SUMMARY

The conclusions to be drawn from the results of these studies are that:

Legume and grass silages, when properly made and fed, produce milks with higher yellow color, finer flavor and greater resistance toward the development of oxidized flavor than do corn silage, beet pulp or dried citrus pulp.

Alfalfa silage is almost equal to spring pasture in putting yellow color into milk and is equal to or better than pasture in producing milk of fine flavor and high resistance toward the development of oxidized flavor.

Beet pulp and citrus pulp are about equal so far as color, flavor and resistance to oxidation are concerned with the exception that a bitter flavor frequently appeared in the milk while the cows were being fed citrus pulp.

The differences in the effects on color, flavor and resistance to oxidation between molasses grass silage and phosphoric acid grass silage are not considered significant.

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THE RELATION OF CERTAIN FACTORS TO THE DRYING OF WHEY MIXTURES ON THE ATMOSPHERIC DRUM DRIER

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INTRODUCTION

About 12 per cent of the whey produced in the United States is utilized for the production of lactose, lactic acid, and whey powder. The remainder must be disposed of as animal feed or be wasted. The disposal of this whey constitutes a problem in the cheese manufacturing industry. A portion usually can be returned to the producing farmers for feeding. Unfortunately, they are able to take only a small part of the total, consequently, much of the whey must go down the sewer. Even its disposal as sewage presents a problem in many communities, as a result of its relatively high solids content of approximately six per cent, as compared to the 0.20 per cent solids content of strong sewage (1).

Whey solids have valuable nutritional properties, being completely digestible, high in lactose, calcium, and phosphorus contents. Although whey solids are low in protein, the protein present is chiefly albumin, which is an excellent supplement to the proteins of the cereals. Of greater nutritional significance than this is the vitamin value of whey. Supplee (2) reports that experimental data have shown the following factors to be present in whey: riboflavin (lactoflavin, vitamin B_2, or G), thiamine (vitamin B), vitamin B_6, prothrombin (vitamin K), and provitamin D. Whey is recognized today as one of the richest sources of riboflavin. Heiman and Carver (3) have shown that a sample of neutralized dried whey contained 10–20 per cent more vitamin G than a sample of dried skim milk. Jukes and Richardson (4) also have recently reported dried whey to be an excellent source of riboflavin. The presence of these dietary substances makes dried whey an excellent supplement to the grains and grain by-products, for use in feeding swine and poultry, as grains tend to be a poor source of riboflavin.

Ways are being developed to use whey solids for human food but the principal recovery of these valuable materials, for the present at least, seems to lie in the direction of animal feeds. Conditioning whey for economical storage, transportation and handling requires the removal of water comprising about 94 per cent of the raw material. Usual methods of drying at present are by the spray process, the atmospheric drum two-stage process, and on the regular atmospheric drum drier.[1] The first two methods require

Received for publication August 24, 1940.

[1] Author's note: Most drying processes are patented. Information concerning the patent status should be secured before using these procedures commercially.

large scale operation and are of little value to the small cheese maker unless he can haul his whey to a central drying plant. The last method uses equipment of a size suitable for small plant operation but certain difficulties are encountered when whey alone is applied to the drums. The heat from the drums has a tendency to caramelize the lactose and to form a sticky, plastic, gummy mass, and, instead of being easily removed by the scraper knives in the form of a thin film, it continuously rolls up on the edges of the knives in the form of a gummy mass which drops into the conveyors. The latter often becomes clogged as a result of this gummy condition. Thus, for efficient operation of the drier, it is necessary to add some material to the whey which will form a sheet on the rolls.

This study has been concerned with some of the fundamental principles applying to the problems of drying whey by the atmospheric drum process, and particularly with the study of the properties of different combinations of whey and film-forming material which yield a satisfactory sheet when scraped from the drums.

EXPERIMENTAL PROCEDURE

Experimental samples were prepared on a laboratory atmospheric drum drier[2] by mixing the film-forming material with liquid whey of various types in the minimum quantities that would form a satisfactory sheet on the machine. The samples were analyzed for total acidity, hydrogen-ion concentration measured as pH, lactose content, and nitrogen content.

Acidities were determined on the liquid before drying by titrating with standard alkali, and pH values were obtained on the dry material reconstituted with water using a Leeds and Northrup quinhydrone pH indicator. Lactose was estimated by the ferricyanide reduction method of Cole (5). In this method the dry material is reconstituted in water, the proteins are precipitated with colloidal iron, and the filtered lactose solution is titrated against boiling alkaline potassium ferricyanide using methylene blue as an internal indicator. Nitrogen was determined by the Kjeldahl method using a semi-micro apparatus.

EXPERIMENTS WITH MIXTURES OF SKIM-MILK SOLIDS AND WHEY

Skim-milk solids in various forms are frequently available for mixing with whey before drying. Therefore, mixtures which dried satisfactorily were obtained using liquid skim milk, condensed skim milk, and dried skim milk mixed with rennet whey or acid-type cottage cheese whey of various original acidities, and after the original acidity had been reduced. The combinations of sweet skim milk and whey of varying developed acidities and the properties of the mixtures are shown in table 1. The lactose/

[2] Loaned through the courtesy of the Buffalo Foundry and Machine Co., Buffalo, New York.

nitrogen ratio is calculated. This ratio is an indication of the effectiveness of the milk protein as a film-forming material.

TABLE 1

Mixtures of sweet skim milk and whey of various acidities which dried satisfactorily

Acidity of whey	Liquid mixture proportions		Lactose content of dried mixture	Nitrogen content of dried mixture	Lactose/ nitrogen ratio	pH
	Whey	Skim				
%	*parts*	*parts*	%	%		
0.11	50.0	50.0	53.33	4.11	12.98	6.40
0.14	37.5	62.5	49.98	4.20	11.89	6.36
0.16	37.5	62.5	49.82	4.25	11.74	6.32
0.18	32.5	67.5	49.88	4.42	11.29	6.33
0.20	30.0	70.0	50.10	4.73	10.58	6.34
0.22	27.5	72.5	49.84	4.59	10.85	6.30
0.25	22.5	77.5	49.03	4.57	10.72	6.26
0.30	20.0	80.0	47.91	4.80	9.98	6.25
0.35	15.0	85.0	46.32	4.91	9.42	6.21

It will be noted that sweet whey and sweet skim milk will dry satisfactorily when mixed in equal parts. This combination has a lactose/nitrogen ratio of almost 13 and a pH of 6.40. As the acidity of the whey increases an increasing amount of skim milk must be added until at 0.35 per cent acidity in the whey 85 per cent of the mixture must be skim milk for satisfactory drying.

The effect of reducing the developed acidity in the whey with sodium hydroxide as related to the amount of sweet skim milk necessary to add was determined and is shown in table 2.

TABLE 2

Effect of neutralizing sour whey to various acidities

Whey acidities		Maximum whey-skim milk mixture that would dry satisfactorily		Lactose contents of dried mixture	Nitrogen content of dried mixture	Lactose/ nitrogen ratio	pH of mixture
Original	After neutralization	Parts of whey	Parts of skim				
				gm./100 gm.	*gm./100 gm.*		
0.35	0.005	50	50	49.44	3.875	12.76	6.75
0.35	0.05	45	55	48.81	4.06	12.02	6.59
0.35	0.10	45	55	48.64	4.075	11.94	6.50
0.37	0.12	35	65	50.94	4.64	10.98	6.41
0.37	0.14	30	70	50.16	4.705	10.66	6.36
0.37	0.16	30	70	50.17	4.70	10.67	6.36
0.37	0.18	30	70	50.26	4.705	10.68	6.31
0.37	0.20	30	70	50.50	4.705	10.73	6.23
0.37	0.22	30	70	50.13	4.72	10.62	6.24
0.37	0.25	25	75	49.49	4.775	10.36	6.18

These results show that developed acidity may be reduced by neutralization to almost entirely restore the original drying properties. This suggests that hydrogen-ion concentration is the limiting factor in the amount of skim milk necessary. To establish whether or not this is correct some mixtures were dried in which the whey was acidified in varying degrees with hydrochloric and sulphuric acids. The results are shown in table 3.

TABLE 3

Acidifying whey to various acidities with acids other than developed lactic acid

Whey acidities		Acid used	Maximum whey-skim milk mixture that would dry satisfactorily		Lactose content of dried mixture	Nitrogen content of dried mixture	Lactose/nitrogen ratio	pH of mixture
Original	After acidification		Parts of whey	Parts of skim				
					gm./100 gm.	*gm./100 gm.*		
0.10	0.10	.....	50	50	52.25	4.135	12.64	6.38
0.10	0.12	HCl	50	50	53.30	4.34	12.28	6.32
0.10	0.14	HCl	40	60	52.96	4.59	11.54	6.32
0.10	0.16	HCl	40	60	51.03	4.28	11.92	6.28
0.10	0.18	HCl	35	65	51.40	4.615	11.14	6.25
0.10	0.20	HCl	30	70	50.92	4.97	10.25	6.25
0.10	0.22	HCl	25	75	49.32	4.82	10.23	6.23
0.10	0.25	HCl	25	75	49.38	4.99	9.90	6.17
0.10	0.30	HCl	25	75	49.71	4.955	10.03	6.12
0.10	0.35	HCl	15	85	49.42	4.95	9.98	6.12
0.10	0.10	.....	50	40	52.25	4.135	12.64	6.38
0.10	0.12	H_2SO_4	50	50	53.30	4.34	12.28	6.28
0.10	0.14	H_2SO_4	40	60	52.74	4.58	11.52	6.28
0.10	0.16	H_2SO_4	35	65	51.86	4.67	11.10	6.24
0.10	0.18	H_2SO_4	30	70	50.85	4.905	10.37	6.24
0.10	0.20	H_2SO_4	30	70	50.83	4.785	10.62	6.23
0.10	0.25	H_2SO_4	25	75	49.91	4.925	10.13	6.13
0.10	0.30	H_2SO_4	20	80	49.13	5.06	9.71	6.16

It is apparent that the results with these mineral acids are similar to those obtained with developed lactic acid. Thus it seems to be fairly conclusive that the deleterious effect of acidity on drying whey and liquid milk mixtures is not the specific effect of lactic acid *per se* but is a general effect caused by increasing hydrogen-ion concentration.

Some trials were made using skim milk condensed to about 32 per cent total solids content. This was mixed with whey of different acidities in the minimum amounts which gave satisfactory drying. The results are shown in table 4.

It can be seen that the skim milk solids in the form of condensed milk are slightly more advantageous to use than as liquid skim milk. This is shown by the higher lactose/nitrogen ratios obtained at lower pH values

TABLE 4

The use of condensed skim milk

Whey acidity	Maximum whey-condensed skim milk mixture that would dry satisfactorily		Lactose content of dried mixture	Nitrogen content of dried mixture	Lactose/nitrogen ratio	pH of mixture
	Parts of whey	Parts of cond.	*gm./100 gm.*	*gm./100 gm.*		
0.095	83	17	56.37	4.11	13.72	6.21
0.12	83	17	56.24	4.07	13.82	6.21
0.13	81	19	55.62	4.175	13.32	6.11
0.14	80	20	55.01	4.26	12.91	6.10
0.15	78	22	53.87	4.225	12.75	6.10
0.17	76	24	53.57	4.34	12.34	6.07
0.205	72	28	52.82	4.44	11.90	6.02
0.24	66	34	51.68	4.67	11.07	6.91
0.31	60	40	50.51	4.78	10.57	5.70

than were obtained with liquid skim milk. The effects of neutralizing whey acidity upon the amount of condensed milk needed were determined and were found to parallel the results obtained with liquid skim milk.

Experiments were also conducted using dry milk mixed with whey. Roller process dry milk could not be used satisfactorily even in very large amounts. Spray process dry milk gave good results when used at the rate of eight pounds to 100 pounds of sweet whey. This is the quantity specified as being necessary by Spellacy (6) in a patented process. Trials were also made using casein. Casein was added to sweet whey in the form of sodium caseinate. The minimum amount of casein that would produce a satis-

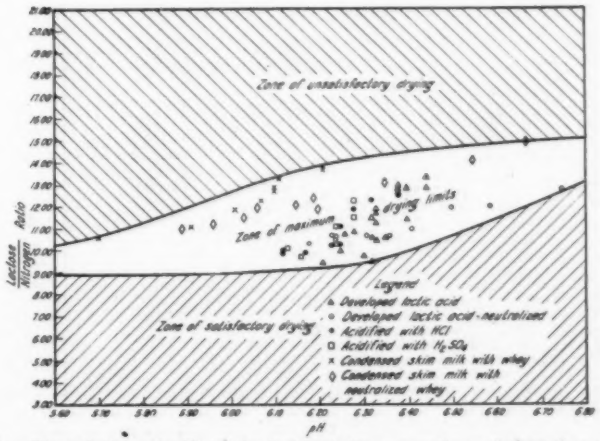

FIG. 1. The relation of pH to the lactose/nitrogen ratio as influencing the drying properties of whey-milk solids mixtures.

factory drying combination with whey was two parts casein per 100 parts of whey. This mixture had a lactose/nitrogen ratio of 10.37.

Precondensing the whey before mixing with the milk solids had no effect in these experiments in altering the lactose/nitrogen ratio for a satisfactorily drying mixture.

The composite picture of the drying properties of whey-skim solids mixtures is shown in figure 1.

EXPERIMENTS WITH CEREALS AND OTHER FEEDS

In many instances it is likely that a sufficient quantity of milk solids may not be available for mixing with whey and suitable animal feed products may be used instead. Mixtures of whey and the following substances were made and the drying properties studied; wheat flour, corn starch, ground oats, ground barley, wheat bran, linseed oil meal, cottonseed meal, soybean meal, ground grain sorghums, ground rice, ground rice hulls, and blood meal. The first four products, namely, wheat flour, corn starch, ground oats, and ground barley were the only ones found to be usable. The results are shown in table 5.

TABLE 5

The use of ceeral products

Cereal	Acidity of whey	Parts of cereal required per 100 parts of liquid whey	Lactose content of dried mixture	Nitrogen content of dried mixture	Lactose/nitrogen ratio	pH of mixture
			gm./100 gm.	*gm./100 gm.*		
Wheat flour	0.10	3.0	50.14	2.275	22.04	6.16
" "	0.30	3.5	44.28	1.855	23.87	4.89
" "	0.40	3.5	43.98	2.185	20.13	4.47
Corn starch	0.12	3.0	47.94	1.50	31.96	6.11
" "	0.20	3.5	44.96	1.48	30.38	5.20
" "	0.45	4.0	40.59	1.275	31.84	4.41
Ground oats	0.12	3.5	44.98	2.105	21.37	5.81
" "	0.20	3.5	43.53	2.16	20.15	5.47
" "	0.38	3.5	42.12	2.095	20.10	4.69
Ground barley	0.12	3.75	44.23	1.945	22.74	6.00
" "	0.20	3.75	40.78	1.865	21.87	5.45
" "	0.38	3.75	41.41	2.02	20.50	4.98

Unlike the milk products, the amount of cereal products required was not much affected by the acidity of the whey. It will be observed also that smaller quantities of solids are needed with these materials than where milk solids are used. This results from the fact that a large portion of the milk solids is lactose which is not a film-forming substance on the drum drier.

Because certain processes for drying whey alone are not as satisfactory when acid-type cottage cheese whey is dried as when used with either ched-

dar cheese whey or with casein whey the materials and quantities reported here have been tried with each kind without any significant difference being apparent.

DISCUSSION OF RESULTS

Observations made during the experimentation showed that those combinations of whey and other materials which yielded a continuous sheet when scraped from the rolls were those in which the added material formed a homogeneous mixture with the whey and thus produced a uniform film on the rolls. The materials which were unsatisfactory on the other hand, did not mix uniformly with the whey and separated on the rolls leaving areas of whey alone and others of added material alone. This is not to be taken to indicate that the satisfactory materials in all cases formed a stable liquid suspension before applying to the drier, because they did not, and most of the cereal products required agitation in order to stay in suspension. There is little ground for speculation as to why some products remained in intimate contact with the whey on the rolls while others of similar composition did not; notable is the failure of ground rice under the conditions of the trials.

The action of the different kinds of whey when dried with some added materials is worthy of comment. It is reported by plant operators that in the drum-drying processes where whey alone is dried that whey from acid-type cottage cheese is much more difficult to dry than whey from cheddar cheese or from casein. During the experiments reported here trials have been made with acid-type cottage cheese whey to compare the quantities of material necessary to add for satisfactory drying with those required for cheddar cheese whey and casein whey. In all trials the results were similar for the different types of whey. The data presented are those of the cheddar cheese and casein whey trials, but the same quantities of materials apply to acid-type cottage cheese whey as well.

SUMMARY

1. Some material which will form a sheet when scraped from the drum must be mixed with whey to dry it satisfactorily on the atmospheric drum drier.

2. Sweet skim milk was found to be such a material. A satisfactory drying combination was obtained by mixing sweet whey and sweet skim milk together in equal amounts.

3. As the titratable acidity of the whey increased, the amount of sweet skim milk required to produce a satisfactory drying combination also increased.

4. Reducing the acidity of sour whey in varying degrees with sodium hydroxide enables one to dry it by using only slightly more skim milk than was required with whey which had developed lactic acid to a corresponding degree of acidity.

5. Sweet whey acidified to various degrees of acidity with hydrochloric or sulphuric acid gave practically the same results as whey which had developed lactic acid to a corresponding acidity.

6. Precondensing the skim milk to be used as the drying agent, enables one to satisfactorily dry a mixture of skim milk solids and whey of a higher lactose/nitrogen ratio at any given whey acidity than was possible when non-condensed skim milk was used.

7. Sour whey neutralized to various degrees of acidity and dried with condensed skim milk, would dry with about as high lactose/nitrogen ratio as whey which had developed lactic acid to a corresponding degree of acidity.

8. Precondensing the whey seemed to have no beneficial effect insofar as the drying properties of mixtures were concerned.

9. It was not possible to dry a mixture of as high a lactose/nitrogen ratio when casein was used for mixing with whey for drying as when other sources of milk solids were used.

10. Spray process powdered skim milk proved satisfactory for mixing with whey for drying when used at the rate of 8 pounds to 100 pounds of sweet whey.

11. Powdered skim milk prepared by the atmospheric drum process did not prove satisfactory, even when used at the rate of 20 pounds per 100 pounds of sweet whey.

12. Ground and sifted cereals, such as corn starch, wheat flour, oats, and barley proved satisfactory as a material to be added to whey when used at the rate of 3–3¾ pounds per 100 pounds of liquid whey. An increase in the titratable acidity of the whey increased slightly the amount of wheat flour or corn starch necessary to produce a satisfactory film, but did not increase the amount of either ground oats or barley required.

13. Similar results have been obtained in all cases with cheddar cheese whey and with acid-type cottage cheese whey.

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American Dairy Science Association Announcements

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November 22, 1940

Officers, Members, and Friends of the
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Honored and pleased that the American Dairy Science Association is to hold its next Annual Meeting at the University of Vermont and State Agricultural College, Burlington, Vermont, June 23–26, 1941, we extend to all a cordial welcome and assure you that we will endeavor to make your visit pleasant and satisfactory in every respect. Come to a state where dairy cattle outnumber people. Burlington is located on beautiful Lake Champlain, only 100 miles from Montreal and in easy driving distance from Ottawa, Quebec and other eastern Canadian points.

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Any member of the Association may submit a title for the program. Members are limited to two papers, of which they are authors or co-authors, unless the extra papers fit into the program being planned. No title will be accepted unless it is accompanied by an abstract. At least one of the authors

of a paper must be a member of the Association. A title and abstract are to be sent to the program committee only when the author or co-author has the intention of presenting the paper.

Publication of abstracts.—The abstracts will be published in the June number of the "Journal of Dairy Science." Please typewrite this plainly since the abstract is a copy for the printer. Leave ample margins and a double space between lines. Prepare the abstract so that it is ready for final publication.

Length of abstract.—The abstract must be short, 500 words or less.

Contents of abstracts.—The abstracts should contain concise statements of (1) the problem under investigation, (2) the experimental method used, and (3) the essential results obtained. Charts, graphs, photographs, and tables will not be accepted.

Time for presentation of papers.—Ten minutes will be allowed for the presentation of each paper.

The titles and abstracts must be in the hands of the program committee not later than April 15, 1940. This committee consists of Glen W. Vergeront, University of Wisconsin, chairman of the program committee of the Extension Section; Walter V. Price, University of Wisconsin, chairman of the program committee of the Manufacturing Section; W. E. Petersen, University of Minnesota, chairman of the program committee of the Production Section; and E. S. Guthrie, Cornell University, general chairman. The titles and abstracts should be sent to the section chairman concerned.

THIRTY-SIXTH ANNUAL MEETING, UNIVERSITY OF VERMONT, BURLINGTON, VERMONT, JUNE 23–26, 1941

Monday, June 23

8:00 A.M.— 5:00 P.M.—Registration.
9:00 A.M.— 3:00 P.M.—Committee meetings.
9:00 A.M.—Board of Directors meeting.
2:00 P.M.— 4:00 P.M.—Dairy products judging conference for coaches and instructors.
3:00 P.M.— 5:00 P.M.—Tour of dairy barns, observation of herds, or continuation of dairy products judging.
8:00 P.M.—Social period.

Tuesday, June 24

9:30 A.M.—11:00 A.M.—Opening session (General program).
11:00 A.M.—12:00 M.—Committee meetings.
1:30 P.M.— 3:30 P.M.—Section program meetings.
3:30 P.M.— 4:30 P.M.—Section business meetings.
8:00 P.M.—Social period.

Wednesday, June 25

9:00 A.M.—11:00 A.M.—Section program meetings.
11:00 A.M.—12:00 M.—Committee meetings.
1:30 P.M.— 3:30 P.M.—Section program meetings.
3:30 P.M.— 4:30 P.M.—Section business meetings.
8:00 P.M.—Social period.

Thursday, June 26

9:00 A.M.—11:00 A.M.—Section meetings.
11:00 A.M.—12:00 M.—Committee meetings.
1:00 P.M.— 3:00 P.M.—Section meetings.
3:00 P.M.— 3:45 P.M.—Section business meetings.
4:00 P.M.— 5:30 P.M.—Business (General program).
7:00 P.M.—Banquet.

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VOLUME XXIV JANUARY, 1941 NUMBER 1

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

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Published in cooperation with
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ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE JOURNAL OF DAIRY SCIENCE

1. **The Influence of Fat in the Making of Cream Cheese.** Z. D. Roundy and W. V. Price, University of Wisconsin, Madison.

Cream cheese was made by the cooked-curd method from various lots of cream containing 8, 10, 12, 14, 16, 18, 20 and 22 per cent fat. The curd was not homogenized. Observations were made of flavor, body, firmness (resistance to penetration), texture, composition, yield and acidity. The quality of cheese made from cream with approximately 18 per cent fat was best in all respects although excellent cheese was regularly made with as little as 14 and as much as 22 per cent fat. Cheese made from cream low in fat tended to lack pleasing flavor, to be dry and crumbly and to lack smoothness while cheese made from the richest cream was sometimes criticized as too rich and too soft and sticky. Increasing fat in the cream caused the cheese made from it to contain more fat and less moisture. About 39 per cent fat and 52 per cent moisture in the cheese gave the most desirable spreading and slicing properties. Yield of cheese per hundred pounds of cream increased while yield per pound of milk fat decreased with increasing amounts of fat in the cream. The acidity (pH) of the cheese was not greatly influenced by the percentage of fat in the cream. It was suggested that variations in quality and composition of cream cheese might be decreased by standardizing cream to a desired ratio of fat to casein. Some differences in quality were associated with changes in fat composition caused by season or feed.

2. **Some Chemical Changes Occurring in Molasses Grass Silage.** C. B. Bender, D. K. Bosshardt and O. F. Garrett, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

A study was made of the rate of fermentation in a silo containing a mixture of field peas and oats preserved with one hundred pounds of molasses per ton of green material.

Periodically, samples were withdrawn through holes drilled in the silo wall. These samples were analyzed for pH, total sugars, reducing sugars, volatile acids, total nitrogen, water-soluble nitrogen, and ammonia nitrogen.

The major portion of the fermentation was found to take place during the first week. The ammonia nitrogen content was negligible indicating that, although there is protein breakdown, the presence of fermentable carbohydrates suppresses ammonia formation.

3. **The Determination of Fat in the Presence of Free Fatty Acids. I. The Mojonnier Test of Mixtures of Free Fatty Acids and Butterfat.** Mortimer P. Starr and B. L. Herrington.

Free fatty acids were mixed together in approximately the ratio found

in cows milk fat. Varying weights of this mixture were added to known amounts of fresh butter oil, previously weighed into Mojonnier extraction flasks. The Mojonnier extraction procedure recovered the neutral fat plus approximately 24 per cent of the added mixture of free acids regardless of the quantity of free acid present.

4. **Blood Sugar and Carbon Dioxide Combining Power of Plasma in Relation to Ketosis in Dairy Cattle.** J. F. SYKES, C. W. DUNCAN, AND C. F. HUFFMAN. From the Departments of Physiology, Experiment Station Chemistry, and Dairy Husbandry, Michigan State College, East Lansing.

In a study of ketosis, the total ketones of the blood, blood sugar and carbon dioxide combining power of the plasma have been determined on a large group of mature dairy cattle. Some of these were made at weekly intervals over considerable periods. With one or two exceptions, clinical symptoms of ketosis were not evident. With increasing degrees of ketosis, the blood sugar values progressively decreased although all these values fell within accepted normal limits. The carbon dioxide combining power of the plasma remained within normal limits at all levels of blood ketones which were encountered in this particular group of cattle and showed no consistent variations which could be correlated with the degree of ketosis.

5. **The Effect of Rations Deficient in Phosphorus and Protein on Ovulation, Estrous and Reproduction of Dairy Heifers.** L. S. PALMER, T. W. GULLICKSON, W. L. BOYD, C. P. FITCH AND J. W. NELSON, University of Minnesota, St. Paul.

Abstract appears on pages 567 and 568 of June issue of the JOURNAL OF DAIRY SCIENCE.

5a. **Factors Involved in the Ejection of Milk.** FORDYCE ELY, University of Kentucky, Lexington, AND W. E. PETERSEN, University of Minnesota, St. Paul.

Abstract appears on pages 536 and 537 of June issue of the JOURNAL OF DAIRY SCIENCE.

6. **"The Relationship between Type and Production."** LYNN COPELAND, American Jersey Cattle Club, New York.

The distinguishing characteristics between various breeds of livestock are principally conformation and color. Without such differences, it would be difficult to distinguish one breed of livestock from another of the same species. Standards of type or conformation are important and the value of such standards is universally recognized. In dairy cattle any standard of

type must either be definitely associated with producing capacity or at least not inimical to it.

Studies have been made of the classification ratings on registered Jersey cows comparing the conformation ratings with production records. The correlation between the classification ratings and the production records was found to be $+.307 \pm .024$. The correlation between the type ratings on cows and the type ratings on their daughters was found to be $+.360 \pm .026$, and the correlation between the type ratings on bulls and the ratings on their daughters was found to be $+.39 \pm .05$. No correlation was found to exist between the classification ratings on bulls and the production records on their daughters.

7. **Body Size and Milk Production.** MAX KLEIBER AND S. W. MEAD, Division of Animal Husbandry, College of Agriculture, University of California, Davis, California.

The theory, that total as well as partial efficiency of energy utilization in animals is in general independent of body size, leads to the postulate that productive capacity of animals should be related to the same function of body size as is their fasting katabolism; namely the $\frac{3}{4}$ power of body weight. In contradiction to this postulate Gaines concluded from lactation data that the capacity for milk production is proportional to body weight itself.

In the present article it is shown, why Gaines' theory would lead to an unnecessary complication in the formulation of food utilization in cows. It is also shown that the data used by Gaines support the $\frac{3}{4}$ power rule of productive capacity better than they support Gaines' own idea provided that these data are evaluated according to their statistical weight.

Data on body size and lactation of Jersey and Holstein cows of the University of California herd are presented and it is shown that the relation of body size and lactation capacity cannot be derived on the basis of milk records alone. It is suggested to express the lactation rate per unit for metabolic body size ($kg^{\frac{3}{4}}$) and use these relative rates as a measure for the relative lactation capacity, a characteristic which should be valuable in breeding for efficiency of milk production.

8. **A Supplemental Note on the Correlation between Fat Percentage and Yield of Milk, Milk Fat, Milk Energy.** W. L. GAINES, University of Illinois, Urbana, Illinois.

Formulas are derived expressing the coefficient of correlation (r) for the variables fat percentage (f), milk yield (M), milk-fat yield (F), and milk-energy yield (FCM), in all possible pairs, entirely in terms of f and M. A previous result (Journal of Dairy Science, April, 1940) is confirmed. The formulas are applied to a set of observed data as a working check on their validity and as a basis for interpretation of the biological significance of the r's.

BOOK REVIEWS

9. Mineral Metabolism. ALFRED T. SHOHL, Res. Assn. in Pediatrics, Harvard Univ. Published by Reinhold Pub. Corp., 332 W. Forty-second St., New York. 384 pages, $5.00.

Milk and its products are used primarily as foods. The nutritional fate of the constituents of dairy products is, for the dairy industry in general, not so well known. Knowledge that fats, proteins and carbohydrates furnish energy and build tissue, and that milk contains vitamins and mineral is not enough. Emphasis is passing from mere knowledge of content to include function of the factors in the metabolic processes. The distinct mineral value of milk is recognized by the industry. The theme "A quart of milk per person per day" and the general fortification of milk with vitamin D emphasize the trend of importance attached to *effect* of the constituents. Hardly a process within the body takes place without the qualifying effects of minerals: pulse, nerve and optical response, tissue lubrication and digestion are but random examples. Minerals are not synthesized, they must be provided by the body.

This book "Mineral Metabolism" describes the manifold activities of minerals in life processes. The 14 chapters cover the subjects of mineral composition of the body, secretions, excretions, base, chloride, ammonium and bicarbonate, calcium and magnesium, phosphorous, sulfur, iron, iodine, trace elements, water, anion-cation relationships, and mineral intakes, balances and requirements, and excellent reference lists. The book is written in orderly text-book form, and primarily from the standpoint of normal physiological requirements.

Among the sections of particular interest to the dairy industry profession are: a concise but interesting comparison of cow's milk, the milk of other animals and woman's milk; calcium, its content, its forms, role in mineralization of bone, rickets and tetany; the merit of "alkaline" foods; and mineral intakes and requirements. The minerals in milk are numerous, hence the discussions on the functions, variations and effects of minerals in physiological processes are of importance in providing information about the uses of minerals less frequently discussed.

A distinctive aid is the recognition throughout of the mineral requirements and changes, physiologically, of infancy, childhood and adulthood. Being an intimate secretion, many interesting citations are made of the relation of various minerals to milk secretion, and the content of the minerals in milk and in the body. The language of the book presupposes knowledge of chemistry and physiology, but the text book arrangement, concisely and clearly written, will enable less well informed readers insight into the relation of the minerals of milk and their fate in nutrition. It is

an excellent book for those engaged in any phase of dairy industry activities where information on the minerals and physiological processes is desired.

K.G.W.

10. **The Chemical Composition of Foods.** R. A. McCance and E. M. Widdowson. Univ. of Cambridge. Published by the Chemical Publishing Co., New York. 150 pages, $2.50.

This book is essentially a tabular presentation of the composition of many important foodstuffs. The data is divided into classes of foodstuffs as follows: Cereals and cereal foods (39) Dairy Products (21) Meat Poultry and Game (81) Fish (88) Fruit (77) Nuts (9) Vegetables (74) Sugar, Preserves and Sweetmeats (18) Beverages (15) Beers (6) Condiments (8) Vegetable Fats (2) Cakes and Pastries (37) Puddings (35) Meat and Fish Dishes (12) Egg and Cheese Dishes (7) Sauces and Soups (9). In the tables are included data of water, sugar, sugar and dextrins, total and amino nitrogen, purine nitrogen, protein, fat, available carbohydrate as glucose, calories, Na, K, Ca, Mg, Fe, Cu, P, S, Cl, acid-base balance, and edible matter. The data is computed for both units per 100 grams, and per ounce. Additional data is included on the "available" iron and phosphorous of a number of products. A convenient index is provided. A feature of the book is presentation of data of both raw and cooked food products. The book will be of real practical value to dietitians, nutritionists and those whose work requires knowledge of the chemical composition of foods.

K.G.W.

11. **Refrigeration Data Book,** Vol. 2, 1940. American Society of Refrigeration Engineers.

Refrigeration Applications. This volume consists mainly of entirely new material on the uses, trades, industries or processes to which refrigeration and air conditioning are applied. The contents is divided into seven parts, I. Freezing Processes; II. Refrigeration in Processing; III. Cold Storage; IV. Distribution of Foods; V. Industrial Refrigeration; VI. Comfort Air Conditioning; VII. Industrial Air Conditioning. Chapters covering subjects directly pertaining to the dairy industry or closely related to it are as follows: 2. Theory of Freezing Foods; 3. Preparation for Freezing; 4. Quick Freezing Fruits; 8. Bacteria of Frozen Foods; 10. Ice Manufacture; 11. Vitamins in Refrigeration; 15. Milk Plants; 16. Ice Cream Plants; 18. Butter and Cheese Making; 19. Cold Storage Buildings; 20. Storage Conditions; 21. Controlled Atmospheres; 26. Refrigeration of Eggs; 29. Ozone and Light in Refrigeration; 31. Load Calculations; 32. Foods in Commercial Refrigeration; 34. Locker Plants; 36. Refrigeration of Cars and Trucks; 37. Uses of Dry Ice; 38. Insulation of Storage Rooms.

In those chapters dealing with dairy products details are covered such as, the country cooling station, the market milk plant, regeneration systems, the condensery, under the heading of Milk Plants. Subjects taken up under Ice Cream Plants comprise manufacture of the mix, refrigeration requirements, the ice cream hardening room coils, delivery equipment, plant layout, cost of power and corrosion.

In connection with Butter and Cheese Making the main items are butter manufacturing process, refrigeration loads and temperatures, and cheese factory refrigeration. L.M.D.

BACTERIOLOGY

12. Studies on Streptococci of Bovine Mastitis. L. W. Slanetz and J. Naghski, Univ. of N. H. Agr. Exp. Sta., Durham, N. H. J. Infect. Diseases, *66:* 80–85. 1940.

Of 680 cultures of weakly hemolytic streptococci isolated from cows showing mastitis infection 84.2 per cent were identified as *Str. agalactiae*, 12.2 per cent as *Str. uberis*, 2.2 per cent as *Str. dysgalactiae*, and 1.3 per cent as *Str. fecalis*. Edwards' medium was found satisfactory for the differentiation of mastitis streptococci, for 98.7 per cent of the blue-gray colonies developing on this medium were identified as *Str. agalactiae*. No cultures of *Str. agalactiae* could be isolated from the mammary tissue of 5 cows known to be mastitis-free for two or more lactation periods. W.C.F.

13. Agglutinability of Mastitis Streptococci. W. N. Plastridge, L. F. Banfield, and L. F. Williams, Storrs Agr. Exp. Sta., Storrs, Conn. J. Infect. Diseases, *66:* 202–211. 1940.

All ingredients, especially the kind of peptone, used in preparing nutrient broth had a pronounced influence on the agglutination properties of bovine udder streptococci. A medium of the following composition was best suited for routine production of antigens for use in the slide agglutination test: beef infusion plus 2 per cent Difco-Proteose peptone, 0.4 per cent dextrose and 1 per cent K_2HPO_4, and adjusted to pH 7.4. Reduction of the dextrose content to 0.1 per cent made possible the preparation of usable antigens from most inagglutinable strains.

Cells of a mucoid-rough variant which were unsatisfactory antigens because they occurred in stringy, viscid masses were made specifically agglutinable by shaking in the presence of N/10 NaOH.

Str. dysgalactiae cultures showed a marked tendency toward spontaneous agglutination which was more difficult to overcome than that of either *Str. agalactiae* or *Str. uberis*.

The tendency of cultures to be specifically agglutinable, spontaneously agglutinable, or inagglutinable was found to vary in different herds. W.C.F.

14. The Comparative Values of Certain Mediums for the Isolation of Bovine Tubercle Bacilli. A. G. KARLSON, Mayo Foundation, Rochester, Minn. J. Infect. Diseases, *67:* 1–4. 1940.

Nonglycerinated mediums were more satisfactory than glycerinated for the isolation of bovine tubercle bacilli. The recommended medium was made by the addition of the yolk of one egg to 150 cc. of melted and cooled beef extract agar. W.C.F.

15. Capsule Formation by Members of the *Brucella* Group. W. A. MICKLE, Duke Univ. School of Medicine, Durham, N. C. J. Infec. Diseases, *66:* 271–277. 1940.

Of 27 strains of *Brucella* studied the five which produced demonstrable capsules were isolated from Hodgkin's disease, while the non-capsulated organisms were isolated mostly from cases of brucellosis. The encapsulated strains were not agglutinable in ordinary antiserum, but were highly agglutinable in serums prepared against homologous organisms. These encapsulated strains served as poor antigens *in vivo,* but non-capsulated organisms obtained by cultivation of the capsulated strains in beef extract broth were satisfactory antigens. W.C.F.

16. The Preparation of *Brucella abortus* Plate Antigen and Some Factors Affecting the Sensitivity of the Antigen. M. H. ROEPKE AND C. P. FITCH, Minn. Agr. Exp. Sta., St. Paul, Minn. J. Infect. Diseases, *66:* 17–22. 1940.

Brucella abortus antigens prepared by washing cultures with large amounts of saline and then centrifuging to remove the substances adsorbed from the medium resulted in antigens with much more uniform sensitivities than antigens prepared by washing with small amounts of phenol-saline solution and then heating over a free flame to the simmering point for 10 minutes. W.C.F.

BREEDING

17. Artificial Insemination of Dairy Cows. Pub'd by National Dairy Products Corp., 230 Park Ave., New York, N. Y.

This is a 16 page bulletin designed to answer questions concerning artificial insemination associations.

The bulletin includes a brief history of the development of artificial insemination. It discusses the advantages to be derived from artificial breeding associations and methods of procedure which may be followed in organizing them. It offers specific suggestions about fees and budgets. Some space is devoted to the management and selection of bulls.

The bulletin makes it clear that while artificial insemination associations have great possibilities for good, those interested in organizing them should give all the facts to prospective members and not make unwarranted claims.

C. L. Blackman

BUTTER

18. Preparation and Care of Starter. MICHAEL B. MICHAELIAN, Chicago, Ill. Nat. Butter and Cheese J., *31:* 10, 16. 1940.

Clean, sterile milk containers and equipment are essential for starter propagation. A heat treatment at 180–200° F. for at least 30 minutes is necessary for fresh whole or skim milk to be used for starter. Strict cleanliness and careful inoculation and incubation of the starter at 70° F. are essential details for success. Precautions to observe are listed such as proper handling of glassware, minimizing contamination during heating and cooling, checking and maintenance of proper temperatures at all times, and daily transfers and frequent renewals of cultures. Specific directions are given for each step of carrying mother culture. W.V.P.

CHEESE

19. Value of Salt in Cheesemaking. J. C. MARQUARDT AND L. RESI. N. Y. Agr. Exp. Sta., Geneva. Nat. Butter and Cheese J., *31:* 10, 32. 1940.

Complete results are given of salt analyses made on American styles of cheese exhibited at the 1940 N. Y. State Fair. Exhibits of different styles from some single factories show practically uniform percentages of salt, while salt percentages in exhibits from other factories are somewhat variable. Low salt contents are detrimental in curing while excessive salt is not so serious but it does delay curing. The desirable range of 1.4 to 1.8 per cent salt for this type of cheese can be attained if salting-rate schedules and careful salting practices are followed by makers. The analyses of a total of 193 entries in all styles show that the percentages of salt ranged from 1.0 to 2.2 and averaged, for all entries, 1.56 per cent. W.V.P.

20. Cheese Analysis Offers Suggestions to Limburger Makers. ANONYMOUS. Nat. Butter and Cheese J., *31:* 10, 23. 1940.

Marquardt, Yale and Resi of the N. Y. (Geneva) Agricultural Experiment Station scored and analyzed for salt and moisture 11 samples of Limburger cheese. The samples were obtained at the 1940 New York State Fair and represented the products of half of the factories in the state. The table of scores and analyses shows moisture percentages ranging from 38.7 to 45.2 per cent and percentage of salt from 1.8 to 2.5 per cent. Cheese with 39 per

cent or less of moisture was criticized for dryness. Percentages of salt were normal. W.V.P.

21. **A Practical Quality Program.** E. C. Damrow, Fond du Lac, Wis. Nat. Butter and Cheese J., *31:* 10, 12. 1940.

To improve milk quality herds must be tested for disease, methylene blue and sediment tests must be made at least twice each month by state licensed operators and the results reported to state authorities and to each producer. Each sediment test disc can be divided into three parts and filed with the plant, producer and state. Inferior milk must be tested frequently and any milk failing to stand up 30 minutes in the blue test should be rejected for human food. Four grades, A, B, C, and Not Salable are proposed and a price differential of 5 cents per hundred weight between the top three is urged. Grades A, B, and C should reduce methylene blue in not less than 5½, 2, and ½ hours, respectively, and the first two should show a clean sediment test. Plant operators must make the quality tests and state authorities must do the necessary field work. W.V.P.

CHEMISTRY

22. **Nomograph for Correction of Lactometer Reading and Calculation of Milk Solids.** Lincoln M. Lampert, Dairy Service Lab., State Dept. of Agr., Sacramento, Calif. Ind. Eng. Chem. (Analy't Ed.), *12:* 9, 527. Sept., 1940.

A nomograph is presented whereby lactometer readings on whole milk may be corrected for temperature and the solids-not-fat calculated when the fat test is shown. The nomograph is based on statistical data and tables in the literature and cannot give results more accurate than these data. Its principal advantages lie in its facility and speed and in the fact that it may be printed on a single page. B.H.W.

23. **Lactic Acid as a Component of Synthetic Resins.** J. T. Stearn, B. Makower, and P. H. Groggins, Bureau Agr. Chem. and Eng., U.S.D.A., Wash., D. C. Ind. Eng. Chem., *32:* 10, 1335–1342. 1940.

A detailed investigation of the use of lactic acid in alkyd resins is reported. Glycerol forms a series of esters with lactic acid and any one of these esters is capable of combining further with polycarboxylic acids to form alkyd resins. In synthesizing these resins advantage is taken of the hydroxyl as well as of the carboxyl function of this acid. These three-component resins compare favorably with the two-component alkyds and the extent to which lactic acid may be used will depend upon its price. On the basis of raw material costs the conversion of lactic acid to methyl acrylate

cannot compete favorably with the synthesis of the acrylate from either ethylene or ethylene oxide. B.H.W.

24. **The Determination of Manganese in Organic Material Containing Large Amounts of Calcium and Chlorides. The Determination of Manganese in the Mouse and in Milk.** THOMAS W. RAY, Dept. of Biochem., Marquette University Medical School, Milwaukee, Wis. J. Biol. Chem., *134:* 677. 1940.

An essential feature in the method consists in removing the chlorides with a minimum amount of sulfuric acid, thus preventing the formation of calcium sulfate. Filtration, therefore, becomes unnecessary and the danger of losing manganese in the process of filtering and washing is eliminated. The manganese content of skimmilk (one sample reported) was found to be 0.0023 mg. per 100 cc. V.C.S.

25. **The Coupled Oxidation of Carotene and Fat by Carotene Oxidase.** JAMES B. SUMNER AND ROBERT J. SUMNER, Dept. of Zoology, Cornell Univ., Ithaca, N. Y. J. Biol. Chem., *134:* 531. 1940.

The bleaching of carotene by carotene oxidase takes place very slowly unless a small amount of fat is present. If an optimal quantity of fat is added, carotene is bleached with great rapidity. Highly unsaturated fats like hempseed oil or soy bean oil are more effective than olive oil or butter. V.C.S.

DISEASE

26. **Handbook on Mastitis.** Production Advisory Committee, International Assn. Milk Dealers. The Association Bull. Int. Assn. Milk Dealers, 32nd year: 18, 3–11. 1940.

It has been estimated that 30 per cent of the dairy cows of the country are affected by mastitis with a loss of 17 per cent of the production of the industry. The streptococci group of bacteria is responsible for 90 per cent of the cases. Manner of infection of a cow, predisposing factors and spread within the herd are treated. Symptoms are described and methods of control and prevention are suggested. In control, the following are especially important; health of herd replacements, identification of mastitis animals in the herd, isolation and disposal of infected cows and practice of good herd management and sanitation. Present knowledge advances no cure for chronic mastitis although the disease can be controlled in most herds. The detailed suggestions are practical and the handbook is intended for distribution by a milk plant to its patrons. E.F.G.

27. **The Resazurin Test for Detecting Mastitis.** R. R. RUSSELL, T. PALMER-JONES, AND G. M. MOIR, Dept. of Agr. Laboratories, Wallace-

ville, N. Z. Extracted from the New Zealand J. of Sc. and Technology, *21:* 3A, 161–167. 1939.

Extensive tests made by these authors indicate that the resazurin test, hitherto used mainly for milk grading is of value in the diagnosis of mastitis. The test, which is comparatively simple, rapid, and economical, agrees well with cultural and microscopic methods. Because the color changes given by the test are due to the activity of both reductase and catalase enzymes, its results are more accurate than those yielded by pH indicators. The test can be completed in about an hour, and very bad cases may often be detected in a few minutes. The method of conducting the test, which is that described by Moir for milk grading, is given in detail. P.H.T.

FEEDS AND FEEDING

28. Vitamin Content of Distillers' By-Products. C. S. Boruff and A. F. Langlykke, Hiram Walker & Sons, Inc., Peoria, Ill., and Simon Black, Univ. of Wis., Madison, Wis. Ind. Eng. Chem., *32:* 9, 1237–1239. 1940.

Methods of preparation, chemical composition and vitamin content of distillers' residues useful as a dairy and poultry feed are discussed. Dried distillers' solubles are high in vitamin B complex, containing 26 to 40 micrograms of riboflavin per gram, as well as being a good source of factor W. Dried screenings are low in vitamin content. Composite distillers' grains contain only small amounts of vitamins A and D but 13.3 to 15 micrograms of riboflavin and 1.0 to 1.3 I.U. of vitamin B_1 per gram as well as sufficient chick antidermatitis factor to protect chicks at a feeding level of 30 to 40 per cent. B.H.W.

29. Vitamins for the Eyes. Anonymous. Milk Dealer, *29:* 11, 62. 1940.

A brief discussion of night blindness and how it is corrected promptly when vitamin A is added to the diet. An eye disease recently announced by the U. S. Public Health Service, caused by deficiency of vitamin G, is also briefly discussed. C.J.B.

FOOD VALUE OF DAIRY PRODUCTS

30. Assessment of the Level of Nutrition: Tests for Vitamin C on Groups of Poorly Fed and Well-fed School-Children. Leslie J. Harris, Nutritional Lab., Univ. of Cambridge and Medical Res. Council. Lancet, *239:* 259–263. 1940.

The simplified saturation test for the level of nutrition with respect to vitamin C was employed in a study of thirty-five boys at an elementary school in a poor district and twenty-nine boys in an institution where the

diet was exceptionally good. Fourteen of the poor-class children were found to be below standard, five showing a relatively severe deficiency, whereas none of the well-fed boys was below standard.

The validity of the saturation tests is discussed. J.F.C.

31. The Effect of Casein and a Casein Digest on Growth and Serum Protein Regeneration. ARTHUR J. MUELLER, KENNETH S. KEMMERER, WARREN M. COX, JR. AND S. T. BARNES, Mead Johnson and Co., Evansville, Ind. J. Biol. Chem., *134:* 573. 1940.

The object of this work was to determine whether a pancreas extract digest of casein, consisting largely of amino acids, would promote as rapid growth and be as effective in the regeneration of serum protein as the unhydrolyzed protein.

Rat feeding trials showed that per gram of nitrogen ingested casein gave 9.75 grams gain in weight, whereas on the same basis the enzymic digest of casein gave 11.9 grams gain in weight. It was evident in all comparisons that the digest of casein was nutritionally equal or superior to casein itself.

In the case of dogs the average potency value obtained for the digest of casein was slightly lower than that for the casein itself but were equal in the regeneration of serum protein. V.C.S.

ICE CREAM

32. Flavor and Dealer Analysis. Special Bull. No. 64, Int. Assn. of Ice Cream Mfrs., Washington, D. C. Sept., 1940.

The Flavor and Dealer Analysis is based on reports of 221 ice cream plants representing 1938 sales of 51, 476, 230 gallons. For 1938 the sales by flavors were as follows:

Vanilla	51.26 per cent	Maple	1.07 per cent
Chocolate	16.36 " "	Cherry	1.01 " "
Strawberry	7.95 " "	Pineapple	.91 " "
Butter Pecan	3.67 " "	Coffee	.62 " "
Peach	1.46 " "	Other flavors	15.69 " "

The survey reveals that there has been an increase in the number of flavors of ice cream in recent years, but the sale of the three standard flavors of vanilla, chocolate and strawberry has remained about the same.

The principal dealers, according to volume of ice cream sales, are drug stores, restaurants, confectionery stores and grocery stores. Through these four outlets 72.58 per cent of the total volume of ice cream is sold. Only 19.6 per cent of the companies reporting sold ice cream exclusively. For those reporting the sale of other products than ice cream, ice cream sales accounted for 60.7 per cent of the total volume of all sales. M.J.M.

33. The Gravity Froster. CLARENCE BIRDSEYE. Refrig. Eng., *40:* 5. Nov., 1940.

The author gives details of operation of a surface contact continuous

freezing apparatus designed to fit into any food plant production line to deliver quickly frozen products in bulk for packaging in large containers. The objects to be covered in the design of the gravity froster were speed in freezing, sanitation, protection of the product from mechanical injury, and the production of one which would keep well during prolonged storage. Other items of design incorporated in the apparatus were reliability of operation, large output per square foot of floor space occupied, versatility, use of the highest freezing temperatures compatible with the desired capacity and speed of freezing, portability, and low cost operation from standpoint of overhead and use of refrigeration with maximum efficiency. This has been accomplished by introducing foods, either solid such as vegetables and fruits, or liquids like milk and cream, or fruit juices to a refrigerated metal surface on which they are quickly and progressively frozen and from which they are being continually scraped and moved along the surface by flexible scrapers. Liquids are delivered from the froster in a slush-like consistency which permits of their being compactly packed into final package containers of any size by means of automatic packaging equipment. It is claimed that the products are cold enough so that large ice crystals will not be formed in subsequent hardening.

Thawed gravity frozen milk shows no coagulation, will produce a normal cream rise, and cream skimmed from the reliquefied milk does not oil off when put into hot coffee. Heavy cream and sherbet mix emerge as a very smooth slush and do not suffer material grain growth during further hardening operations.

Fluid products may be deaerated before entering the apparatus and while in it may be held under an atmosphere of CO_2, nitrogen or other inert gas. Frozen thus the slushed products include a minimum of oxygen when placed in the containers, and therefore keep exceptionally well during storage.

A 20 station gravity froster has a minimum capacity of 3200 lb. per hour of 40 per cent butterfat cream employing a freezing temperature of + 5° F.

L.M.D.

MILK

34. Checking the Sanitary Quality of Milk and other Dairy Products. H. H. WEISER, Dept. of Bact., Ohio State Univ. Milk Dealer, *29:* 11, 36. Aug., 1940.

The following observations on the Burri slant technique in checking quality are given:

The Burri agar slant technic offers a simple and an inexpensive method to detect the general types of micro-organisms in various dairy products.

Composition of the medium used in this method may be varied to suit the needs of different types of bacterial growth requirements as well as

indicating the relative numbers of organisms present in the product examined.

This method offers a better differentiation of colonies than pour plates because all the colonies are on the surface.

Burri technique usually gives lower bacterial counts than pour plates because clumps are less completely broken up.

This method offers many possibilities and indicates that it may be an acceptable routine procedure adaptable for studying the number and types of organisms in milk as well as in other dairy products. It also offers a practicable and inexpensive method for the cultural examination of mastitis milk. C.J.B.

35. Problems in Adopting the Milk Cow. JOHN L. WILSON, U. S. Dept. of Agr. Milk Dealer, *29:* 11, 42–58. 1940.

A brief history of the fluid milk industry is followed by a discussion of government control, cost of service, canned milk, dairy industry stabilizers, some butter facts, the federal price-stabilizing program, and by-product utilization.

In looking ahead the author states that various trends and tendencies are apparent. The center of the milk-producing region moves southward and westward. Commercial dairying advances moderately in the South, and farmers in the West milk more cows as population increases. Dairy products on the table assume an improved quality and increased variety. Safety of the milk supply continues to be stressed, but inspection systems may be coordinated. More emphasis will be placed on products utilizing all the constituents of milk rather than the fat alone, and in government control there appears in the distance a coordination of producer, dealer, and consumer relationships toward a bigger and better dairy business. C.J.B.

36. Browning of Autoclaved Milk. Chemical Factors Involved. J. P. KASS AND L. S. PALMER, Div. of Agr. Biochem., Univ. of Minnesota, St. Paul, Minn. Ind. Eng. Chem., *32:* 10, 1360–1366. Oct., 1940.

The results of an investigation to determine the chemical reactions which characterize the caramelization of lactose are reported and an explanation for the development of brown color in heated milk is given. Previous investigators have not agreed as to whether the brown color was due to a caramelization of lactose or to a condensation of lactose with milk proteins.

The discoloration is accompanied by the development af acidity, pronounced fall of optical activity, comparatively slight loss of copper-reducing ability and an appreciable but constant conversion of lactose to ketoses or substances not oxidized by sodium hypoiodite. When allowance is made for the specific nature and effect of each buffer, the trend of the caramelization reaction is the same for all buffers, and the coloration developed is a

logarithmic function of the optically inactivated lactose. This loss of activity and the discoloration is a complex function of buffer concentration and duration of heating and is directly proportional to the initial concentration of lactose. Formaldehyde inhibits coloration but does not prevent optical inactivation while bromination decolorizes the lactocaramel as well as the brown protein precipitated from autoclaved milk.

Casein adsorbs caramel according to the Freundlich isotherm and the extent of adsorption is determined by the pH of the sol and is complete at pH 11. The brown color of heated milk is ascribed to the caramelization of lactose by, and the adsorption of the lactocaramel on, the milk caseinates. No stoichiometric bifunctional reaction between the aldose and amino groups forming definite lactose-protein compounds is involved. B.H.W.

37. The Acidity Test: Effect of Concentration of Indicator on the Apparent (Titratable) Acidity of Milk. H. BARKWORTH. Dairy Ind., *5:* 9, 288. Sept., 1940.

The author has analyzed the data of other investigators relative to the concentration of phenolphthalein in the titratable acidity tests of milk.

It is demonstrated statistically that phenolphthalein should be used in a concentration of 80 milligrams per 100 ml. of milk in order to obtain the desired color reaction at pH 8.3. Lower concentrations tend to give the color change at a higher pH while concentrations greater than 80 mgs. per 100 ml. of milk result in slightly lower pH endpoint.

A 2 per cent solution of phenolphthalein is to be preferred over weaker solutions but it is pointed out that the strength of the solution is not as important as the concentration of dye added to the sample to be titrated

D.V.J.

38. A Comparitor for the Resazurin Test. J. G. DAVIS AND S. B. THOMAS. Dairy Ind., *5:* 9, 244. Sept., 1940.

A recently developed instrument for reading Resazurin tests is described and methods for its use discussed. Two types have been developed, one of which may be used only for Resazurin comparisons while the other may be adopted to other colorimetric comparisons.

The instrument known as the Lovibond Comparitor consists of a bakelite box in which is housed an interchangeable bakelite disc. Glass color standards are fitted into the disc which is revolved in front of one of two apertures cut in the face of the comparitor. The test sample and a blank solution are placed in test tubes or cells which are fitted into a compartment below the viewing apertures.

The authors state that the instrument has been tested for some time in a number of laboratories and that workers had little difficulty in matching colors. D.V.J.

39. The Resazurin Test. GEORGE M. MOIR, Dairy Division's Lab., Wallaceville, New Zealand. Reprinted from the New Zealand Dairyman.

The use of a dye called resazurin for milk grading was first suggested by some German workers in 1929. Since then it has been studied by various investigators, who found it to have important advantages over the methylene blue test. Three advantages are: it provides a series of color changes instead of a single one; only one hour is required as compared with five hours with the methylene blue test; and resazurin is much more easily reduced by abnormal milks. The method and materials needed for testing milk and cream are explained fully in this article. P.H.T.

40. Lipolytic Activity in Milk and Cream. D. C. ROAHEN AND H. H. SOMMER, Dept. of Dairy Industry, Univ. of Wisconsin, Madison, Wis. Dairy World, *19:* 5, 22. Oct., 1940.

This is a short discussion of lipolytic action in milk and cream based on experimental results. Among other things, it was found that the optimum pH for lipolysis is 8.4 to 8.6; that the action is only one-third as great at pH 6.25 as at pH 6.6; and that the reduced lipolysis in cream separated at a temperature of 110° F. is not explained by the assumption that under this condition more lipase goes into the skimmilk since the skimmilk also exhibited less lipolytic activity than was found with lower temperatures of separation. F.J.D.

PHYSIOLOGY

41. The Effect of Pituitary Stalk Section upon Reproductive Phenomena in the Female Rat. EDWARD W. DEMPSEY AND UNTO U. UOTILA, Dept. of Anatomy and Physiology, Harvard Medical School. Endocrinology, *27:* 573. 1940.

The pituitary stalk was sectioned in 27 adult female albino rats and following the operation 2 to 3 months' time was permitted to elapse in order to assure complete recovery from the operation. It was observed that reproductive cycles, ovulation, mating, pregnancy, and lactation were normal after section of the pituitary stalk in the rat while the depressing action of cold upon the reproductive cycle and the ovary was abolished by pituitary stalk section. Hypophyseal deficiency was sometimes observed after pituitary stalk section and the authors were of the opinion that it was the result of inadvertent damage to the anterior pituitary or its blood supply and did not represent a physiological effect of denervation. Such deficient pituitaries were sometimes activated following the injection of pregnancy urine extracts. Evidence was presented which indicates that the pituitary stalk mechanism modifies the rate of production of thyrotropic and gonadotropic hormones in response to certain emergency situations. R.P.R.

42. Gonadotropic Action of Pituitaries from Pregnant Cows. A. NALBANDOV AND L. E. CASIDA, Dept. of Genetics, Univ. of Wisconsin. Endocrinology, *27:* 559. 1940.

Individual cattle pituitaries were quantitatively assayed by the implantation of acetone dried pituitary powder into rats at 31 days of age and the injection of aqueous suspensions of the powder into chicks at 14 days of age. From early to late pregnancy there was a steady and significant decrease in gonadotropic activity. Correlations between pituitary weight and fetus length were not significant, thus indicating no significant change in pituitary weight during pregnancy. The difference in gonadotropic content of pituitaries from cows carrying female fetuses and cows carrying male fetuses was not statistically significant. Rat recipients showed predominant luteinization in their ovaries when implanted with pituitaries from cows in early pregnancy. Follicular stimulation was prevalent in the ovaries of rats implanted with pituitaries from cows in late pregnancy. R.P.R.

MISCELLANEOUS

43. Operation, Maintenance and Inspection of Boilers. J. F. HUNT, The Hartford Steam Boiler Inspection and Insurance Co., New York, N. Y. Milk Dealer, *29:* 11, 35, 82–84. 1940.

The author points out that in connection with the safe operation of boilers and other pressure vessels, the first thing to consider is a safely designed installation. The second consideration is that safety appliances be properly installed and kept in good operating condition. It is emphasized that installation and safety appliances should meet the A.S.M.E. boiler construction code.

External and internal corrosion are also discussed. Internal corrosion is divided into pitting, general corrosion, and grooving; pitting being the most troublesome and attributed to the presence of oxygen. The remedy given is the use of proper deaerating processes. Grooving is a localized form of corrosion which seems to form most readily at points where stresses are more or less localized. General internal corrosion attacks the metal more or less uniformly and is brought about by impurities of the feedwater.

Furnace explosions and the installation and maintenance of super-heater units are also discussed. C.J.B.

44. Streamlining the Cost of Record Keeping. J. S. SEIDMAN, C.P.A., Seidman and Seidman, New York, N. Y. Milk Dealer, *29:* 11, 38, 78. 1940.

The author suggests several short cuts which will avoid waste of time and effort in record keeping. The first suggestion is to weed out all useless forms and reports. This is followed by suggestions to eliminate the rewriting

nuisance. The latter is applied to purchases, cash records, sales and accounts receivable records, and statements to customers. Suggestions are also given for simplifying the handling of bank statements and payrolls. C.J.B.

45. Research Uncovers Potential Sales. A. C. DAHLBERG, N. Y. Agr. Exp. Sta., Geneva, N. Y. Milk Dealer, *29:* 11, 106–110. 1940.

A discussion of how new and improved products may uncover potential sales. The subject is discussed under the following headings: Improvement through Feeding, Improved Color, Milk as an Exclusive Diet, Fats in the Diet, Calcium Assimilation, Dairy Industry Research, and Nutritional Research Changed. The author concludes with the statement that, "The dairy industry needs to give more attention to the promotion of both nutritional and industrial research by current methods." C.J.B.

46. Chlorine Sterilization of Dairy Plants. A. L. PROVAN. Dairy Ind., *5:* 9, 240. Sept., 1940.

A comprehensive review of the literature on chlorine sterilization. All of the common chlorine compounds are discussed and compared on the basis of their action. The effect of pH, temperature and organic contamination on germicidal action are discussed as well as the problems of metal corrosion and development of flavor taints. D.V.J.

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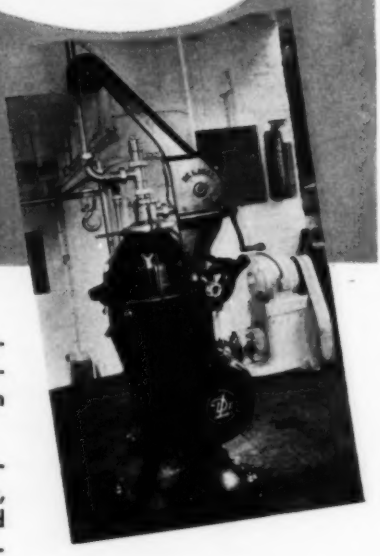

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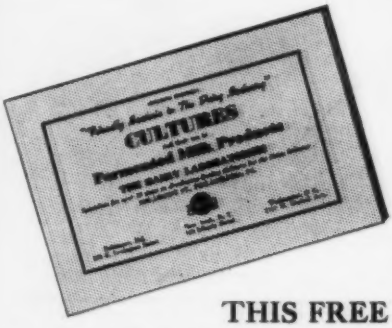

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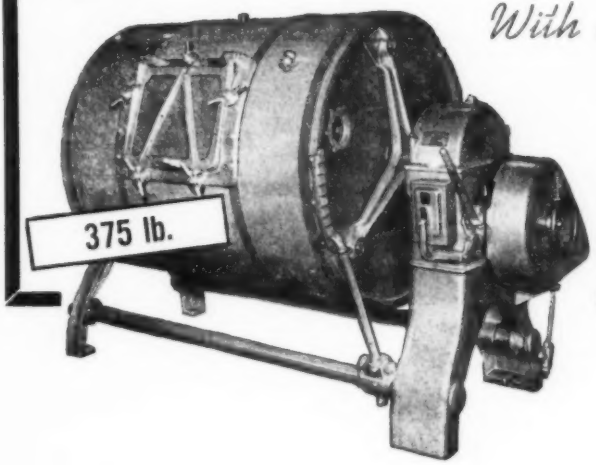

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